JOURNAL OF AGRICULTURAL RESEARCH

VOL. 58 WASHINGTON, D. C., JANUARY 15, 1939 No. 2

THE MODIFICATION OF FLOWER STRUCTURE BY ENVIRONMENT IN VARIETIES OF LYCOPERSICUM ESCULENTUM[1]

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INTRODUCTION

The relation of flower structure to fruit setting in the tomato (*Lycopersicum esculentum* Mill.) has never received adequate consideration. Undoubtedly this has been due in part to the assumption that the relationship as to size, particularly length, between the two essential organs of the flower is very narrowly limited by the genetic constitution of the particular strain or variety concerned. The extent to which the lengths, relative to each other, of the two essential organs might be modified by environment calls for further study.

During the course of work recently reported concerning the effect of carbohydrate deficiency upon meiosis and spermatogenesis in the tomato (*10*)[3] evidence was obtained indicating that the length of the stamens and pistil relative to each other was considerably modified in Marhio, the one variety studied. In consequence of this and the observations of Burk (*6*), a study was made of the flowers of a number of varieties in order to find: (1) To what extent within a variety the relative length of stamens and pistil is modified by environment; (2) what differences occur between varieties in this respect; and (3) what relationship exists between the induced modification and the subsequent fruit-setting behavior within a given variety. This paper reports the data relating to the first and second of these considerations.

STRUCTURE OF THE FLOWER

The tomato flower is characterized by several stamens, typically six, attached by short filaments to the corolla. The anthers are long, adnate, connivent, and form a cone or tube surrounding the pistil. The pistil consists of a syncarpous ovary, an elongated style, and a more or less flattened stigma.

Burk (*6*) in 1930 pointed out that in the variety Bonny Best the length of the pistil appeared to vary in accordance with the length of the photoperiod and the light intensity. With 8 hours of light he found that the pistils were much longer than the stamens, but with 16 hours the pistils did not extend above the tips of the anthers. He also stated that the flowers of the English variety Princess of Wales had styles shorter than the stamens in both photoperiods.

[1] Received for publication March 19, 1938.

[2] The writer wishes to express his thanks and appreciation to G. H. Stringfield, associate agronomist of the Ohio Agricultural Experiment Station and agronomist of the Bureau of Plant Industry, U. S. Department of Agriculture, for advice and aid in the statistical phases of the problem.

[3] Italic numbers in parentheses refer to Literature Cited, p. 116.

METHODS AND MATERIAL

The data and observations presented in this paper were taken from several series of plants grown from 1933 to 1937, inclusive. All plants except those of series 1 were grown in the greenhouses of the Ohio Agricultural Experiment Station at Wooster, Ohio; those of series 1 were grown in one of the greenhouses of the John Innes Horticultural Institution, London, England.

The treatments used were designed to induce within the plants a mild to moderate deficiency of reserve carbohydrates. In order to bring this about the plants were supplied with an abundance of nitrogen and were grown with the other environmental conditions favorable for a rate of amino acid synthesis sufficiently rapid to result in little accumulation of carbohydrate reserves.

As indicated in table 1, the plants were grown during various seasons and in various media.

TABLE 1.—*Season of the year and media in which plants were grown, 1933-37*

Series No.	Season	Media in which plants were grown
1	April to July 1933	Pots; manure-loam soil mixtures.
2	May to July 1934	Do.
3	March to June 1935	Ground bed and pots; manure-loam soil mixture and sand-drip culture.
4	April to July 1936	Ground bed and pots; manure-loam soil mixture.
5	March 1937	Pots; manure-loam soil mixture.

The seed from which most of the plants were grown was obtained from commercial firms in Columbus, Ohio; Philadelphia, Pa.; Grand Rapids, Mich.; Edinburgh, Scotland; and Reading, England. Seed of certain varieties also was furnished by W. A. Huelsen of the University of Illinois, I. C. Hoffman, and L. J. Alexander of the Ohio Agricultural Experiment Station, and by the Ontario Horticultural Experiment Station, Vineland Station, Ontario, Canada.

The flowers of more than 50 varieties and strains were examined over the 5-year period. The procedure followed has usually been to examine the flowers on the first six clusters of a number of plants of each variety. In this way variations between flowers within the same cluster as well as between succeeding clusters were noted.

PRESENTATION OF DATA

SERIES 1

The plants of series 1 were the same as series 3 and 4 of an earlier investigation (*10*) in which the effect of carbohydrate deficiency upon meiosis and spermatogenesis in the tomato was studied. The plants were of the Marhio variety, a pink-fruited selection of Marglobe. Two manure-loam soil mixtures were used for potting the plants. One mixture consisted of one part of manure to three parts of a rich potting soil and the other consisted of three parts of manure to one part of soil. The plants in the three-quarter manure mixtures were further divided into two lots, one receiving ammonium sulphate and the other no nitrogen-carrying fertilizer.

A special effort was made to grow these plants in a vigorously vegetative condition from the time they were transplanted as young

seedlings until they had produced several flower clusters. That they made such growth is indicated by figure 1, *A*. After the plants were potted into the manure-soil mixture, particularly those in the three-quarter manure-soil mixture, they grew vigorously and produced

FIGURE 1.—*A*, Typical vigorously vegetative plant of series 1 just after potting into manure-soil mixture; *B*, typical vigorously vegetative plants of series 1 after several flower clusters had been produced; *C*, extent of fruit development in first (*a*) and second (*b*) cluster of typical plant of series 1, showing hand-pollinated, selfed fruit with nearly full seed content; *D*, typical flowers (*a*, *b*) from first cluster showing stigma practically flush with and tightly clasped by tips of the stamens and (*c*, *d*) typical flowers of the third cluster with pistils enclosed well within the staminal cones.

thick, succulent stems. The leaves were very dark green, succulent, and somewhat malformed. Figure 1, *B*, shows two plants after they had grown under treatment in the one-quarter manure-soil mixture for some time. The plants had received similar treatment to those of series Q reported upon by Kraus and Kraybill (*13*), and exhibited

similar growth characteristics. They were also similar in appearance to the plants of series A studied by Nightingale, Schermerhorn, and Robbins (*15*).

The first flower of the first cluster reached anthesis on May 12 during the long daylight period of latitude 51° at London. All flowers of all clusters reached anthesis and produced anthers of a deep-yellow color.

The flowers of the first cluster of all plants either failed to develop into fruits or developed into small irregular-shaped fruits with few or no well-developed seeds. Figure 1, *C*, *a*, *b*, shows the first and second clusters of a typical plant. A fruit with a fair complement of seeds was produced only when a flower was self-pollinated by actual transference of the pollen from the anther to the stigma, as was done with the fourth flower of the second cluster (fig 1, *C*, *b*). Examination of the flower structure showed that in all cases the tips of the stamens were flush with the stigma, in other words, the pistil and stamens were of equal length (fig. 1, *D*, *a*, *b*). Furthermore, the stamens tightly clasped the style, thus preventing pollen from sifting down and over the surface of the stigma.

The same situation, that is, pistils and stamens of equal length, occurred in the flowers of the second cluster as they reached and passed anthesis. Tapping the flower clusters, the common commercial practice, had no beneficial effects on fruit setting. Only actual transference of the pollen was effective. The equal length of the pistil and stamens and the fact that the stigma was tightly clasped by the tip of the staminal cone was responsible for the failure of the flowers in these two clusters on these vigorously vegetative plants to develop into fruits under the long daily light period.

On the other hand, tapping the flowers of the third cluster was effective in inducing self-pollination. In figure 1, *D*, *c*, *d*, are shown two flowers of the third cluster with pistils shorter than the stamens. The flowers of all the remaining clusters also set fruit freely.

SERIES 2

Plants of 15 varieties were potted in 8-inch pots containing a manure-loam mixture of one part manure to three parts of a rich potting soil. The seed had been sown in April 1934, and the plants were put under treatment in late May. During the period from June 6 to June 30, observations were made on the relative length of the pistil and stamens. The observations are presented in table 2.

The position of the stigma in relation to the tips of the stamens on June 6 as compared to June 30 shows a significant change in all but one variety, Break O' Day. This variety alone showed the stigmas well within the staminal cone, not only in the flowers of the first cluster, but also in those of the third and fourth. In the other varieties the length of the pistil relative to that of the stamens was less in the flowers of the third and fourth clusters than in those of the first.

On June 6 the flowers of the first cluster of Bonny Best, Earliana, and Greater Baltimore had styles protruding considerably beyond the stamens, but on June 30 the pistil and stamens were of about equal length. The flowers of Beauty and Ponderosa had long, protruding styles on June 6 in the first cluster, and in the flowers of the clusters produced later the styles still extended slightly beyond the tips of the stamens. Marglobe and Marhio showed the stigma only slightly

within the staminal cone on June 6, but well within on June 30. Globe had a slightly protruding style on June 6, but the stigma and tip of the stamens were flush with each other on June 30. The shorter length of the pistil relative to that of the stamens on June 30 as compared with June 6 was concomitant with a slower rate of growth of the plants, that is, reduced rate of organic nitrogen synthesis and consequent greater carbohydrate accumulation at the end of the month of June than at the beginning.

TABLE 2.—*Classification of the 15 varieties of tomato in series 2 on the basis of the position of the stigma in relation to the tips of the stamens on June 6 and on June 30, 1934*

FIRST CLUSTER, JUNE 6

Stigma well within staminal cone.	Stigma slightly within staminal cone.	Stigma flush with tips of stamens.	Stigma slightly above tips of stamens.	Stigma well above tips of stamens.
Break O' Day.	Potentate.[1] Marglobe. Marhio.	Pritchard. Tuckswood.[1 2]	Blair Forcing. Globe. Riverside Favorite.[1]	Beauty. Bonny Best. Earliana. Greater Baltimore. Ponderosa. Veals.[1]

THIRD AND FOURTH CLUSTERS, JUNE 30

Break O' Day. Marglobe. Marhio. Potentate.	Blair Forcing. Earliana. Pritchard. Tuckswood.	Bonny Best. Globe. Greater Baltimore. Riverside Favorite. Veals.	Beauty. Ponderosa.	

[1] Seed of this variety was furnished by the Ontario Horticultural Research Station from a supply obtained from W. S. Bewley of the Research Station, Cheshunt, Herts, England.

[2] The seed firm of Gebrüder Dippe A. G., Quedlinburg an Harz, Germany, lists the variety Tuckswood as synonymous with Kondine.

During early July the styles of the flowers of the subsequently produced clusters of Globe, Bonny Best, Tuckswood, and Riverside Favorite were enclosed well within the staminal cone.

By late July some of the flowers of the upper clusters of these varieties again had pistils extending well beyond the tips of the stamens. Considerable fruit production had occurred on the lower clusters and the maximum temperature within the greenhouse during the day had risen considerably. In consequence, a localized carbohydrate deficiency had developed in the upper part of the plant adjacent to the developing flower clusters. Nitrates were found microchemically in both the upper and lower leaves and stems during this period. This condition of protruding styles is commonly observed in the flowers of the upper cluster of the Globe and Marhio varieties in the greenhouse in late October and early November and is associated with heavy withdrawal of carbohydrate material by the developing fruits of the lower clusters.

SERIES 3

Series 3 consisted of nine varieties grown from March to June 1935. The plants were divided into three lots, one of which was grown in sand-drip culture, another in pots, and the third in a ground bed.

In the pots the soil was composed of three parts of loam to which a considerable amount of well-rotted manure had been added just before the planting of the previous fall crop.

Beginning with the opening of the flowers of the first cluster, measurements were taken by means of a vernier caliper of the length of the stamens, style, and distance the stigma extended above or below the tips of the stamens (called the pistil length minus length of stamens). Frequently the length of the ovary was measured in order to check the values obtained indirectly by the other measurements. Several flowers of each of the first eight clusters of representative plants of each variety in all treatments were measured as above indicated. Data given are from only two plants of each variety, however, since these data are representative of those obtained from the other plants (table 3). The discussion in each variety is concerned only with the flowers of a cluster which were measured.

The regression of pistil length minus length of stamens (the distance the pistil extended above or below the tips of the stamens) on length of the pistil was obtained for each variety. This regression is useful in showing that as the pistils were longer they tended more and more to approach or exceed the stamens in length. If, however, pistils and stamens varied in length entirely independently of each other, the longer pistils might still have the same tendency relative to length of stamens. The question, Is it variation in length of pistil or in length of stamens that principally affects the degree of pistil exsertion? is, therefore, not answered by these regressions. But if either pistils or stamens contributed the more greatly to increasing flower length and if with increasing length of these organs the degree of pistil exsertion was greater, it would seem a reasonable inference that the organ contributing the more was the one principally associated with the degree of pistil exsertion. To explore this possibility, the pistil-stamens average length, one-half (pistil length plus stamens length), was calculated for each flower and regressions were computed of both pistil length and of stamens length on this value. It is recognized that in such regressions the y variable is necessarily correlated with x, since in part they are measures of the same thing. But the point of interest is in the comparison between the two regressions; namely, pistil length on the pistil-stamens average length, and length of stamens on the same average length of pistil and stamens. In other words, the interest is in the relative contributions of the pistil and stamens to change in the average length of the pistil and stamens. These regressions are presented in connection with the discussion of each variety.

Variety Blair Forcing

The pistils of the flowers of the first and second clusters with one exception extended above the tips of the stamens (table 3). The maximum distance was 1.8 mm. Beginning with the third cluster and continuing through the succeeding clusters, the pistils with only four exceptions were enclosed within the staminal cone. In two flowers the pistils and stamens were of equal length. Figure 2, *A*,[4] shows the relative position of these organs in three flowers of the second cluster on April 11, and figure 2, *B*, shows two flowers from the fourth cluster on April 24. The position of the pistil within the staminal cone on the latter date is clearly indicated.

[4] In this and succeeding figures of series 3 the flowers shown have frequently been taken from plants for which data are not presented in table 3.

TABLE 3.—*Measurements of the length of the ovary, style, pistil, and stamens, and of the distance the stigma was located above or below the tips of the stamens, for tomato varieties in series 3, March to June 1935*

BLAIR FORCING

Order of flower cluster	Medium in which plant was grown and order of flower in cluster	Length of—				Distance of stigma above (+) or below (−) tips of stamens	Medium in which plant was grown and order of flower in cluster	Length of—				Distance of stigma above (+) or below (−) tips of stamens
		Ovary	Style	Pistil	Stamens			Ovary	Style	Pistil	Stamens	
		Millimeters	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*		*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*
	Ground bed:						Pot:					
1	2	0.8	7.7	8.5	9.4	−0.9	1	1.8	9.0	10.8	9.2	+1.6
	3	2.0	7.8	9.8	9.3	+.5	2	3.0	8.6	11.6	10.6	+1.0
							4	1.1	8.7	9.8	9.0	+.8
2	1	1.7	9.5	11.2	9.4	+1.8	3	.9	10.0	10.9	9.8	+1.1
	2	1.4	8.2	9.6	9.1	+.5	4	1.6	9.2	10.8	10.8	+.8
3	5	.5	8.7	9.2	9.6	−.4	1	.7	8.2	8.9	9.6	−.7
							2	1.5	8.0	9.5	10.2	−.7
4	1	1.0	8.2	9.2	9.8	−.6	4	.6	7.9	8.5	9.0	−.5
							5	.8	7.6	8.4	8.8	−.4
5	1	1.5	8.2	9.7	10.2	−.5	2	1.5	8.9	10.4	10.4	.0
	2	.6	7.7	8.3	8.0	+.3						
	3	1.3	7.8	9.1	9.3	−.2						
	4	.7	8.2	8.9	9.3	−.4						
6	2	.9	9.1	10.0	10.3	−.3	1	.9	8.0	8.9	10.0	−1.1
	3	1.4	8.8	10.2	10.2	.0	2	.8	8.9	9.7	10.5	−.8
	5	1.1	9.0	10.1	9.1	+1.0						
7	4	.8	8.2	9.0	9.3	−.3						

TABLE 3.—*Measurements of the length of the ovary, style, pistil, and stamens, and of the distance the stigma was located above or below the tips of the stamens, for tomato varieties in series 3, March to June 1935*—Continued

BONNY BEST

Order of flower cluster	Medium in which plant was grown and order of flower in cluster	Length of—				Distance of stigma above (+) or below (−) tips of stamens	Medium in which plant was grown and order of flower in cluster	Length of—				Distance of stigma above (+) or below (−) tips of stamens
		Ovary	Style	Pistil	Stamens			Ovary	Style	Pistil	Stamens	
	Ground bed:	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	Sand-drip culture:	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*
1	3	2.1	9.3	11.4	10.0	+1.4	3	2.0	8.5	10.5	9.6	+0.9
	4	.7	9.6	10.3	9.4	+.9						
2	2											
	3	2.4	8.2	10.6	9.4	+1.2	1	2.8	7.5	10.3	10.3	.0
	4	2.0	8.4	10.4	9.2	+1.2	2	1.1	8.0	9.1	9.8	−.7
3	1	1.7	7.7	9.4	8.4	+1.0						
		1.6	7.9	9.5	10.5	−1.0	1	1.3	8.2	9.5	9.5	.0
4	1						2	1.7	8.8	10.5	10.5	.0
	2	1.1	8.4	9.5	10.0	−.5						
		.9	7.6	8.5	9.6	−1.1	1	1.0	9.0	10.0	10.8	−.8
5	1						2	1.8	9.0	10.8	10.0	+.8
	2	2.0	7.8	9.8	10.5	−.7	3	.9	9.1	10.0	10.5	−.5
	3	.8	7.5	8.3	8.8	−.5						
		2.2	7.6	9.8	10.4	−.6	1	2.5	8.7	11.2	11.2	.0
6	1						2	1.9	8.9	10.8	10.8	.0
	2	2.6	8.2	10.8	10.5	+.3	3	1.8	8.7	10.5	10.8	−.3
	3	1.1	9.4	10.5	9.5	+1.0						
	4	1.6	9.0	10.6	10.1	+.5	1	2.1	8.2	10.3	10.3	.0
	5	1.4	8.8	10.2	9.2	+1.0	2	1.9	8.5	10.4	10.4	.0
		1.1	8.3	9.4	9.4	.0	3	2.4	8.5	10.9	10.6	+.3
7							4	1.4	7.5	8.9	9.4	−.5
8	1											
	2	1.9	8.0	9.9	9.6	+.3	1	1.3	8.2	9.5	9.8	−.3
	3	1.2	8.0	9.2	8.7	+.5						
		1.5	7.4	8.9	8.5	+.4						

BREAK O' DAY

	Ground bed: [1]						Ground bed: [2]					
1	3	1.1	7.7	8.8	11.6	−2.8	2	2.6	9.0	11.6	11.6	0.0
							3	1.5	8.7	10.2	10.5	−.3
							4	.7	8.0	8.7	10.7	−2.0
2	1	1.8	8.6	10.4	10.8	−.4	1	2.0	8.5	10.5	10.5	.0
	2	1.5	8.0	9.5	10.2	−.7	3	2.5	7.6	10.1	11.5	−1.4
	3	1.6	7.4	9.0	10.8	−1.8						
3	2	1.7	7.5	9.2	12.0	−2.8	1	1.6	7.2	8.8	11.6	−2.8
	3	.8	8.0	8.8	11.6	−2.8	2	1.3	6.4	7.7	10.7	−3.0
4	1	1.4	8.0	9.4	10.8	−1.4	1	1.9	8.7	10.6	11.1	−.5
	2	2.4	7.4	9.8	11.2	−1.4						
	3	2.1	8.0	10.1	11.1	−1.0						
5	1	1.8	8.0	9.8	10.4	−.6						
	2	1.4	7.5	8.9	10.3	−1.4						
	3	1.7	7.3	9.0	10.5	−1.5						
6	3	.7	7.6	8.3	10.1	−1.8						
	4	.8	7.6	8.4	10.2	−1.8						

EARLIANA

	Ground bed:						Sand-drip culture:					
1	1	2.8	8.7	11.5	10.0	+1.5	5	2.5	8.8	11.3	10.4	+0.9
	3	2.4	9.6	12.0	10.2	+1.8						
	4	1.9	9.7	11.6	10.1	+1.5						
2	2	2.2	9.3	11.5	10.1	+1.4	6	1.0	9.0	10.0	10.0	.0
	3	1.2	9.0	10.2	9.5	+.7						
	4	2.0	8.5	10.5	10.5	.0						
3	1	1.0	8.9	9.9	11.9	−2.0	6	1.5	9.0	10.5	10.5	.0
	3	.8	9.6	10.4	9.4	+1.0						
4	3	2.8	9.0	11.8	11.8	.0	4	1.7	7.8	9.5	11.0	−1.5
5	1	1.1	8.9	10.0	10.3	−.3	2	1.4	9.7	11.1	11.5	−.4
	2	1.8	9.2	11.0	11.0	.0						
6	1	1.4	8.3	9.7	9.3	+.4	3	2.4	7.0	9.4	10.4	−1.0

[1] Plant No. 1.
[2] Plant No. 2.

TABLE 3.—*Measurements of the length of the ovary, style, pistil, and stamens, and of the distance the stigma was located above or below the tips of the stamens, for tomato varieties in series 3, March to June 1935*—Continued

GLOBE

Order of flower cluster	Medium in which plant was grown and order of flower in cluster	Length of—				Distance of stigma above (+) or below (−) tips of stamens	Medium in which plant was grown and order of flower in cluster	Length of—				Distance of stigma above (+) or below (−) tips of stamens
		Ovary	Style	Pistil	Stamens			Ovary	Style	Pistil	Stamens	
		Millimeters	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*		*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*
1	Ground bed:						Pot:					
	2	2.8	7.9	10.7	10.7	0	1	2.7	7.7	10.4	8.9	+1.5
	3	1.9	7.5	9.4	9.6	−.2	3	1.5	7.5	9.0	9.0	.0
	4	1.5	7.6	9.1	9.8	−.7						
2	2	1.0	8.0	9.0	9.5	−.5	1	1.2	7.2	8.4	8.4	.0
	3	.6	7.4	8.0	9.0	−1.0	2	1.2	7.0	8.2	8.2	.0
							3	1.0	7.0	8.0	8.7	−.7
3	1	1.0	7.0	8.0	9.2	−1.2	1	2.0	7.0	9.0	9.0	.0
	2	1.0	7.0	8.0	9.5	−1.5	2	.9	6.8	7.7	8.2	−.5
	3	2.6	6.6	9.2	10.0	−.8						
4	1	1.7	7.5	9.2	9.6	−.4	1	1.7	7.5	9.2	9.2	.0
	2	1.5	7.0	8.5	9.0	−.5	2	1.6	7.4	9.0	9.4	−.4
	3	1.3	7.2	8.5	9.2	−.7	3	2.2	7.2	9.4	10.0	−.6
5	1	1.4	7.6	9.0	9.0	.0	1	1.3	6.2	7.5	8.7	−1.2
	2	1.4	7.8	9.2	9.5	−.3	2	.8	7.0	7.8	8.4	−.6

GREATER BALTIMORE

1	Ground bed:					Pot:							
	3	2.1	9.0	11.1	9.6	+1.5	4	1.4	8.6	10.0	8.9	+1.1
2	1	1.7	9.4	11.1	10.0	+1.1	3	1.0	8.7	9.7	9.0	+.7
	2	2.0	9.2	11.2	9.8	+1.4	4	1.2	9.4	10.6	9.6	+1.0
	3	2.0	9.5	11.5	9.8	+1.7						
3	2	2.0	9.0	11.0	9.5	+1.5	1	.7	7.6	8.3	7.8	+.5
	3	1.0	8.6	9.6	10.4	−.8	2	2.1	8.0	10.1	10.1	.0
							3	1.3	7.7	9.0	9.6	−.6
4	1	1.8	8.3	10.1	11.4	−1.3	3	.7	8.0	8.7	9.0	−.3
	2	1.0	8.0	9.0	9.3	−.3	4	.8	8.0	8.8	9.1	−.3
	3	1.2	8.2	9.4	10.2	−.8						
5	1	1.7	9.2	10.9	10.9	.0	1	1.5	9.0	10.5	10.5	.0
	2	2.8	9.0	11.8	10.4	+1.4	2	2.2	8.8	11.0	9.2	+1.8
	3	1.8	10.5	12.3	10.1	+2.2	3	2.3	8.7	11.0	9.6	+1.4
6	1	2.8	9.6	12.4	10.0	+2.4	1	1.4	8.6	10.0	10.0	.0
	2	1.6	8.4	10.0	10.0	.0	2	1.2	8.0	9.2	9.2	.0
	3	2.0	8.7	10.7	9.4	+1.3						
8	1	1.4	8.5	9.9	8.2	+1.7						

TABLE 3.—*Measurements of the length of the ovary, style, pistil, and stamens, and of the distance the stigma was located above or below the tips of the stamens, for tomato varieties in series 3, March to June 1935*—Continued

LORILLARD

Order of flower cluster	Medium in which plant was grown and order of flower in cluster	Length of—				Distance of stigma above (+) or below (−) tips of stamens	Medium in which plant was grown and order of flower in cluster	Length of—				Distance of stigma above (+) or below (−) tips of stamens
		Ovary	Style	Pistil	Stamens			Ovary	Style	Pistil	Stamens	
	Ground bed:	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	Sand-drip culture:	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*
1	1	2.9	8.5	11.4	9.9	+1.5	1	0.9	7.8	8.7	8.2	+0.5
	2	1.6	8.5	10.1	9.1	+1.0	2	1.7	8.6	10.3	8.7	+1.6
	3	1.7	8.3	10.0	9.4	+.6	3	1.5	7.8	9.3	9.0	+.3
	5	2.4	8.8	11.2	9.6	+1.6	4	2.0	7.4	9.4	9.6	−.2
2	1	1.9	8.2	10.1	10.1	.0	1	1.0	8.0	9.0	9.0	.0
	2	2.6	8.3	10.9	10.0	+.9	5	1.3	8.0	9.3	8.7	+.6
3	1	3.4	7.8	11.2	10.8	+.4	1	2.4	7.5	9.9	10.0	−.3
	2	1.2	7.8	9.0	9.0	.0						
	3	1.8	7.8	9.6	10.1	−.5						
4	1	2.3	7.7	10.0	10.8	−.8	1	.7	8.2	8.9	9.6	−.7
	2	.5	8.3	8.8	9.4	−.6	2	1.6	8.0	9.6	10.0	−.4
	3	.5	8.2	8.7	9.4	−.7						
5	3	1.5	9.3	10.8	8.8	+2.0	1	1.0	7.6	8.6	8.6	.0
	4	1.2	8.2	9.4	9.4	.0	2	1.7	7.5	9.2	9.5	−.3
	5	1.1	8.8	9.9	8.9	+1.0	3	1.1	7.9	9.0	9.0	.0
							4	2.2	7.2	9.4	9.4	.0
7	1	1.6	8.0	9.6	9.6	.0	1	1.9	8.0	9.9	9.9	.0
	2	2.3	7.7	10.0	10.0	.0	2	1.2	8.2	9.4	9.1	+.3
							3	1.4	8.2	9.6	9.6	.0
							4			8.3	9.2	−.9

MARHIO

1	Pot: 4	2.4	8.2	10.6	11.8	−1.2	Sand-drip culture: 1	1.9	6.2	8.1	9.3	−1.2
							2	1.1	7.0	8.1	9.0	−.9
2	1	1.8	7.7	9.5	11.3	−1.8	4	2.0	6.9	8.9	10.6	−1.7
3	1	1.9	7.5	9.4	10.9	−1.5	4	.7	7.3	8.0	8.7	−.7
							5	1.2	8.4	9.6	10.2	−.6
4	1	1.8	7.6	9.4	10.7	−1.3	2	2.0	7.0	9.0	11.0	−2.0
							3	1.6	7.4	9.0	11.0	−2.0
							4	1.8	7.4	9.2	11.2	−2.0
5	1	1.0	7.2	8.2	9.9	−1.7	1	1.8	9.2	11.0	10.0	+1.0
	2	1.5	6.5	8.0	9.6	−1.6	2	1.8	8.1	9.9	11.1	−1.2
							3	1.3	7.8	9.1	10.4	−1.3
							4	1.6	7.1	8.7	9.5	−.8
6	2	1.5	6.5	8.0	9.4	−1.4	1	1.4	6.6	8.0	9.9	−1.9
							2	.7	7.5	8.2	9.4	−1.2

PONDEROSA

1	Ground bed: 1	2.2	11.0	13.2	9.4	+3.8	Sand-drip culture: 1	2.8	10.4	13.2	9.5	+3.7
	2	2.2	10.6	12.8	9.8	+3.0	2	2.5	7.7	10.2	10.2	.0
	4	2.4	10.8	13.2	10.0	+3.2	3	1.0	7.7	8.7	10.2	−1.5
2	1	2.8	10.4	13.2	10.4	+2.8	1	1.5	10.7	12.2	9.5	+2.7
	2	2.2	10.7	12.9	10.7	+2.2	2	2.2	10.5	12.7	10.0	+2.7
	4	2.0	9.4	11.4	10.9	+.5	3	1.7	11.1	12.8	9.8	+3.0
							4	2.0	10.6	12.6	10.0	+2.6
3	1	3.5	10.0	13.5	10.5	+3.0	1	2.7	11.5	14.2	10.5	+3.7
	2	3.5	10.7	14.2	9.5	+4.7	2	2.4	11.4	13.8	11.0	+2.8
	3	3.7	9.5	13.2	11.0	+2.2	3	1.8	11.0	12.8	10.2	+2.6
	4	2.0	11.0	13.0	9.5	+3.5						
	5	3.1	10.4	13.5	10.7	+2.8						
4	1	3.4	10.3	13.7	11.5	+2.2	1	2.9	10.8	13.7	11.5	+2.2
	2	2.1	9.2	11.3	10.8	+.5	2	2.3	9.5	11.8	10.8	+1.0
5	2	2.6	10.2	12.8	10.5	+2.3						
	3	1.5	9.8	11.3	8.7	+2.6						

Figure 3, *A*, shows the regression of pistil length minus length of stamens on pistil length. It is to be noted that the former changed 0.71 mm for each 1-mm increase in pistil length. In figure 4, *A*, there are presented the regressions of both length of pistil and length of stamens on the pistil-stamens average length. It was evident that the regression of pistil length was greater than that of length of stamens, since in the former the increase was 1.22 mm and in the latter 0.62 mm to a change of 1 mm in the pistil-stamens average length. This definitely indicated that the change in pistil length was the factor that principally affected either the degree of pistil exsertion or the distance the stigma was located below the tips of the stamens.

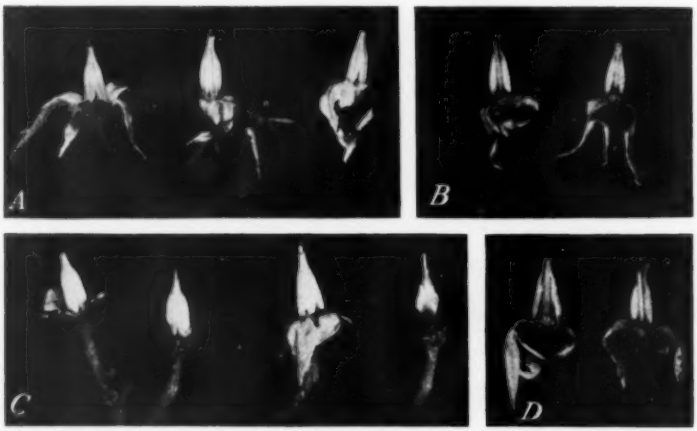

FIGURE 2.—Typical flowers of the second (*A*) and of the fourth (*B*) cluster of the Blair Forcing variety, April 11 and 24, 1935, respectively, and of the second (*C*) and the fourth (*D*) cluster of Bonny Best on the same dates. Note that the pistils in *B* are enclosed within the staminal cone, and that one flower in *D* has pistil and stamens flush, whereas the other flower shows the pistil slightly shorter than the stamens.

VARIETY BONNY BEST

Table 3 also presents data for two plants of the variety Bonny Best, one grown in sand-drip culture and the other in the ground bed. As was found with Blair Forcing, the pistils of the flowers of the first two clusters of the plant in the bed extended well above the tips of the stamens. In the three succeeding clusters the pistils were enclosed within the staminal cones. With the sixth and eighth clusters there occurred a reversal of the relative position of the stamens and pistil, back to that shown in the first and second clusters. Figure 2, *C*, shows flowers taken from the second cluster on April 11 and figure 2, *D*, from the fourth cluster on April 24.

The data for the plant in sand (table 3) did not show the wide variation between succeeding clusters that was shown by the plant in the bed. During the time when the first two clusters were reaching anthesis this plant was not growing in as vigorously vegetative condition as the corresponding plants in the bed. However, in the succeeding clusters the plant remained vigorous and the pistil and

stamens were still either of equal length or the pistil was usually slightly shorter than the stamens.

The regression of pistil length minus stamens length on length of pistil is given in figure 3, *B*. It is to be noted that the former changed 0.59 mm to an increase of 1 mm in pistil length. This value was very similar to that obtained in Blair Forcing. In figure 4, *B*, are presented the regressions of both length of pistil and of stamens on the pistil-stamens average length. Here, again, the value obtained for the regression of pistil length was much greater than the corre-

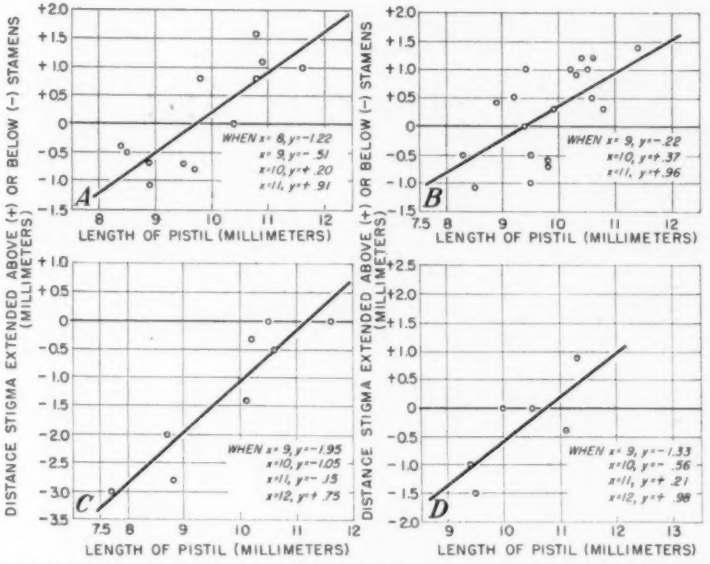

FIGURE 3.—The regression of pistil length minus stamens length (y) on length of pistil (x) in: *A*, Blair Forcing, $b=0.71$ mm; *B*, Bonny Best, $b=0.59$ mm; *C*, Break O' Day, $b=0.90$ mm; *D*, Earliana, $b=0.77$ mm.

sponding value for length of stamens, being 1.33 and 1.08 mm, respectively, for a change of 1 mm in the pistil-stamens average length.

VARIETY BREAK O' DAY

The flowers of the variety Break O' Day showed a marked difference (table 3) from those of the two varieties just discussed. At no time did the pistil extend beyond the tips of the stamens and in only two instances were the stamens and pistil of equal length. In a considerable number of the flowers the stigma was located well within the staminal cone; in fact, as much as 3 mm in an extreme case. Figure 5, *A*, shows flowers taken from the second cluster on April 11, and 5, *B*, shows flowers taken from the fourth cluster on April 24. The pistils are well enclosed within the staminal cones.

Figure 3, *C*, presents the regression of the pistil length minus length of stamens on length of pistil. The former changed 0.90 mm for a change of 1 mm in length of pistil. This value was thus con-

siderably higher than the corresponding ones obtained for Blair Forcing and Bonny Best. In figure 4, *C*, the length of the pistil increased 1.52 mm to a change of 1 mm in pistil-stamens average length. The corresponding value for length of stamens on pistil-

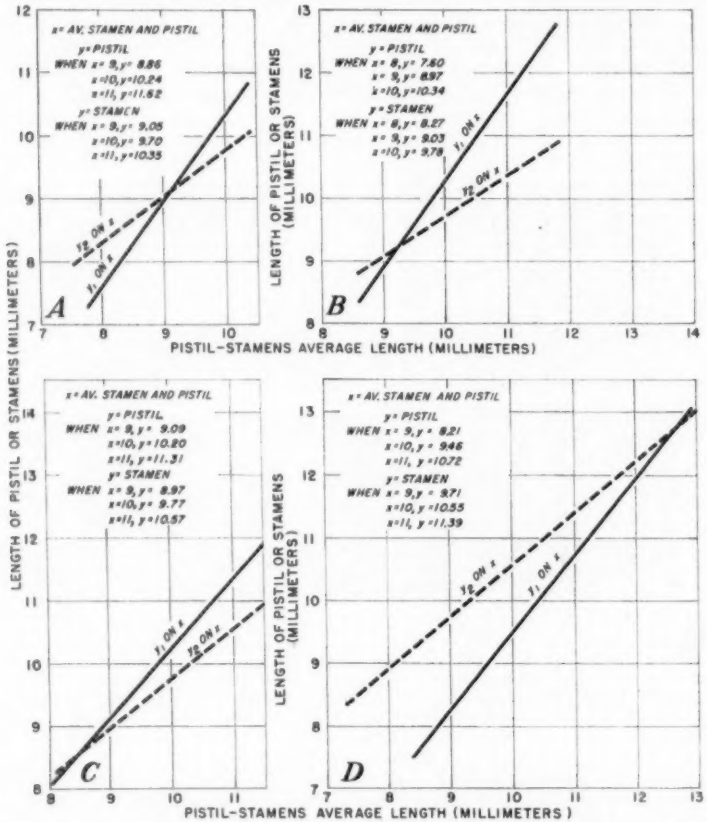
x = AV. STAMEN AND PISTIL
y = PISTIL
WHEN x = 9, y = 8.86
x = 10, y = 10.24
x = 11, y = 11.62
y = STAMEN
WHEN x = 9, y = 9.05
x = 10, y = 9.70
x = 11, y = 10.35
y1 ON x
y2 ON x
A
x = AV. STAMEN AND PISTIL
y = PISTIL
WHEN x = 8, y = 7.60
x = 9, y = 8.97
x = 10, y = 10.34
y = STAMEN
WHEN x = 8, y = 8.27
x = 9, y = 9.03
x = 10, y = 9.78
y1 ON x
y2 ON x
B
x = AV. STAMEN AND PISTIL
y = PISTIL
WHEN x = 9, y = 9.09
x = 10, y = 10.20
x = 11, y = 11.31
y = STAMEN
WHEN x = 9, y = 8.97
x = 10, y = 9.77
x = 11, y = 10.57
y1 ON x
y2 ON x
C
x = AV. STAMEN AND PISTIL
y = PISTIL
WHEN x = 9, y = 8.21
x = 10, y = 9.46
x = 11, y = 10.72
y = STAMEN
WHEN x = 9, y = 9.71
x = 10, y = 10.55
x = 11, y = 11.39
y2 ON x
y1 ON x
D
LENGTH OF PISTIL OR STAMENS (MILLIMETERS)
PISTIL-STAMENS AVERAGE LENGTH (MILLIMETERS)

FIGURE 4.—In each instance the line y_1 on x shows the regression of pistil length on the pistil-stamens average length (x), and the line y_2 on x the regression of the length of stamens on the pistil-stamens average length (x). The b values given are for the first and second line, respectively, as follows: *A*, Data for a plant of Blair Forcing grown in a pot, $b = 1.22$ and 0.62 mm; *B*, data for a plant of Bonny Best grown in the ground bed, $b = 1.33$ and 1.08 mm; *C*, data for a plant of Break O' Day grown in the ground bed, $b = 1.52$ and 0.37 mm; *D*, data for a plant of Earliana grown in sand, $b = 1.12$ and 0.76 mm.

stamens average length was only 0.37 mm. This indicated that pistil length was even more closely correlated in this variety with distance of stigma below the tips of stamens than in Blair Forcing and Bonny Best.

VARIETY EARLIANA

Table 3 presents the data for the Earliana variety. The pistils of the flowers of the first three clusters of the plant in the bed showed

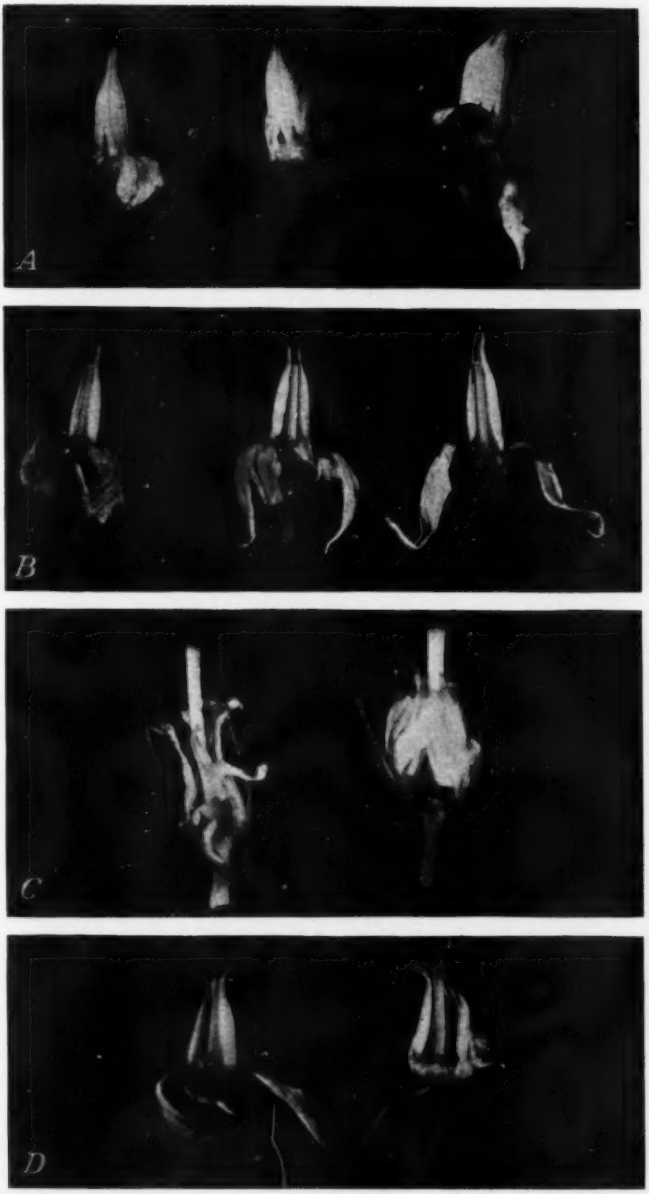

FIGURE 5.—Typical flowers of the second (*A*) and of the fourth (*B*) cluster of the variety Break O' Day, April 11 and 24, 1935, respectively, and of the second (*C*) and the fourth (*D*) cluster of the variety Earliana on the same dates. Note that the pistils in *D* are slightly shorter than the staminal cones.

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a tendency to extend well above the tips of the stamens. The plant in the sand had two flowers with pistils and stamens of equal length and one flower with the pistil extending beyond the stamens. In the succeeding cluster of both plants several flowers had pistils considerably shorter than the stamens, but the remaining flowers tended again to have pistils and stamens of equal length. Figure 5, *C*, shows two flowers taken from the second cluster on April 11, and figure 5, *D*, shows two taken from the fourth cluster on April 24.

Figure 3, *D*, presents the regression of pistil length minus stamens length on length of pistil. The former increased 0.77 mm to an increase of 1 mm in length of pistil. Figure 4, *D*, shows the regressions of length of pistil and of stamens on the average pistil-stamens length. In this variety the value for the pistil was 1.12 mm and for the stamens 0.76 mm for a change of 1 mm in pistil-stamens average length.

Variety Globe

The data for the variety Globe, presented in table 3, show that the pistils of all but two flowers of the plant in the bed were shorter than the stamens. Up to and including the flowers of the third cluster, the pistil became progressively shorter in relation to the length of the stamens. In the following clusters, the fourth and fifth, the pistils, though still within the staminal cones, became progressively longer in relation to the stamens.

The pistil of one flower of the first cluster of the plant in the pot extended 1.5 mm above the tips of the stamens, whereas in the other flower the pistil and stamens were of equal length. Beginning with the second cluster at least one flower to a cluster showed a pistil shorter than the stamens.

The regression of the distance the stigma extended above or below the tips of the stamens on pistil length is presented in figure 6, *A*. The value for the former was 0.58 mm for an increase of 1 mm in length of pistil. In figure 7, *A*, it is to be noted that the pistil length increased 1.37 mm for an increase of 1 mm in the pistil-stamens average length. On the other hand, the corresponding value for length of stamens was 0.65 mm. In these respects this variety was similar to the variety Earliana.

Variety Greater Baltimore

The data for the variety Greater Baltimore are rather similar to those presented for the varieties Blair Forcing, Bonny Best, and Earliana (table 3). The flowers of the first two clusters of plants for which data are presented showed pistils considerably longer than the stamens. The third cluster was transitional and in the fourth cluster the pistils were located within the staminal cones. Again, in the fifth and sixth clusters following a week of little sunshine the pistils extended above the tips of the stamens. In two instances the pistils were 2 mm or more longer than the stamens. Figure 8, *A*, shows two flowers taken from the second cluster on April 11, and *B*, two from the fourth cluster on April 24.

In figure 6, *B*, the regression of pistil length minus length of stamens on length of pistil shows a value of 0.94 mm for a change of 1 mm in length of pistil. Figure 7, *B*, presents the value of 1.38 mm for change in pistil length to 1 mm in pistil-stamens average length,

whereas the corresponding value for length of stamens was 0.75 mm. In this respect the plants of the varieties Earliana, Globe, and Greater Baltimore were similar.

Variety Lorillard

The data for the variety Lorillard, shown in table 3, are similar to those for the variety Greater Baltimore. It was evident that in each of the two plants the relative length of the pistil and stamens changed from a position in which the pistils extended well above the tips of the stamens to the reverse condition. Again, as in several of the preceding varieties, the flowers produced on the uppermost

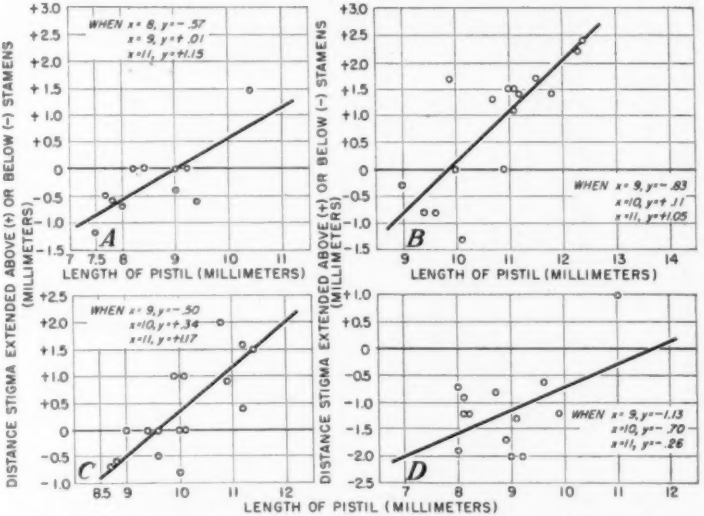

FIGURE 6.—Regression of pistil length minus stamens length (y) on length of pistil (x) in: *A*, Globe, $b=0.58$ mm; *B*, Greater Baltimore, $b=0.94$ mm; *C*, Lorillard, $b=0.83$ mm; *D*, Marhio, $b=0.43$ mm.

clusters possessed pistils extending well above the stamens. Figure 8, *C*, shows typical flowers of the variety taken from the first cluster on April 11 and *D* shows comparable flowers from the fourth cluster on April 24.

Figure 6, *C*, presents the regression of the pistil length minus stamens length on length of pistil. This value was 0.83 mm for an increase of 1 mm in length of pistil. The regression of pistil length on pistil-stamens average length (fig. 7, *C*) shows a value of 1.11 mm for an increase of 1 mm in the pistil-stamens average length as compared to a corresponding value of 0.80 mm for the length of stamens. Thus Lorillard is similar to the three preceding varieties in respect to the regression of pistil length on the pistil-stamens average length.

Variety Marhio

The data in table 3 show that the flowers of the Marhio variety were similar to those of Break O' Day. With one exception the pistils were shorter than the stamens in all flowers measured on the two

plants. Figure 9, *A*, shows three typical flowers taken from the second cluster on April 11. From figure 6, *D*, it is seen that the distance the stigma extended above or below the tips of the stamens increased 0.43 mm for an increase of 1 mm in length of pistil. In figure 7, *D*, are presented the regressions of pistil and of stamens length on the pistil-stamens average length. The value for length

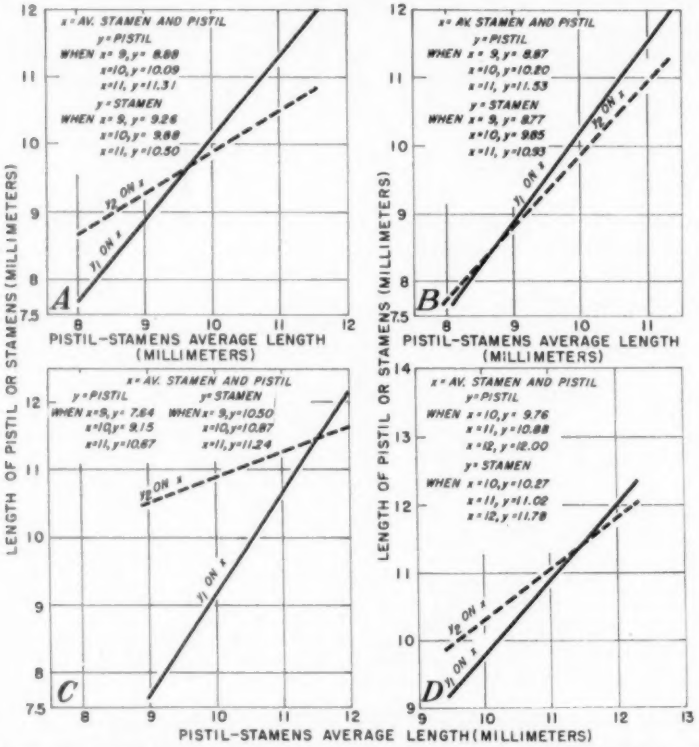
x = AV. STAMEN AND PISTIL
y = PISTIL
WHEN x = 9, y = 8.88
x = 10, y = 10.09
x = 11, y = 11.31
y = STAMEN
WHEN x = 9, y = 9.26
x = 10, y = 9.88
x = 11, y = 10.50
y2 ON x
y1 ON x
A
B
C
D
LENGTH OF PISTIL OR STAMENS (MILLIMETERS)
PISTIL-STAMENS AVERAGE LENGTH (MILLIMETERS)

FIGURE 7.—In each instance the line y_1 on x shows the regression of pistil length on the pistil-stamens average length (x), and the line y_2 on x the regression of the length of stamens on the pistil-stamens average length (x). The b values given are, for the first and the second line, respectively, as follows: *A*, Data for a plant of Globe grown in a pot, $b = 1.37$ and 0.65 mm; *B*, data for a plant of Greater Baltimore grown in sand, $b = 1.38$ and 0.75 mm; *C*, data for a plant of Lorillard grown in ground bed, $b = 1.11$ and 0.80 mm; *D*, data for a plant of Marhio grown in sand, $b = 1.25$ and 0.84 mm.

of pistil was 1.25 mm as compared to 0.84 mm for a change of 1 mm in the pistil-stamens average length.

VARIETY PONDEROSA

With one exception, the pistils of the variety Ponderosa extended above the tips of the stamens throughout the entire period of flowering (table 3). In this respect the variety was in a class by itself. How-

ever, the extent to which the pistil extended above the staminal cone showed considerable variation. With each succeeding cluster it seemed to become progressively shorter in relation to the length of the stamens. Figure 9, *B*, shows typical flowers taken from the second

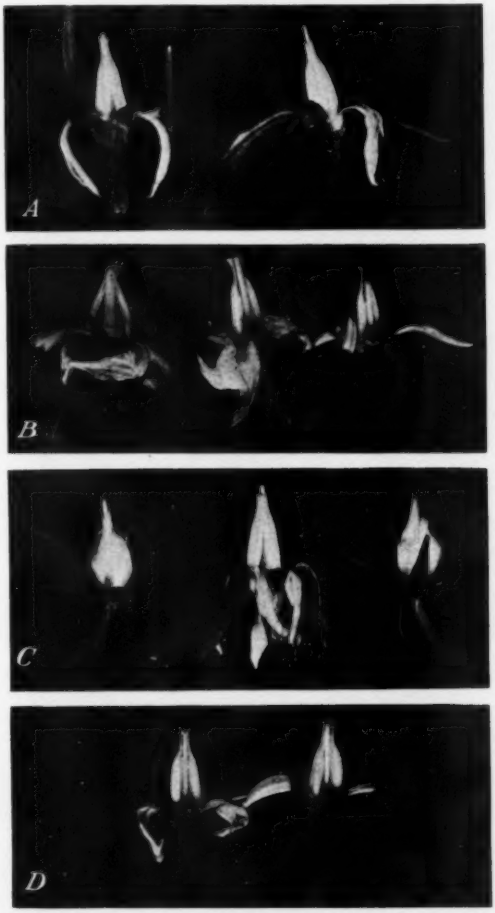

FIGURE 8.—Typical flowers of the second (*A*) and of the fourth (*B*) cluster of Greater Baltimore April 11 and 24, 1935, respectively, and of the first (*C*) and the fourth (*D*) cluster of Lorillard on the same dates. Note that in the flowers shown the pistils and stamens are practically equal in length. Data for flowers in *A* and *B* are not given in table 3.

cluster on April 11; and *C*, flowers from the fourth cluster on April 24.

In figure 10, *A*, the regression of pistil length minus stamens length on pistil length showed a value of 0.86 mm for an increase of 1 mm in length of pistil. Figure 10, *B*, shows the regressions of the length of pistil and of stamens on the pistil-stamens average length. The

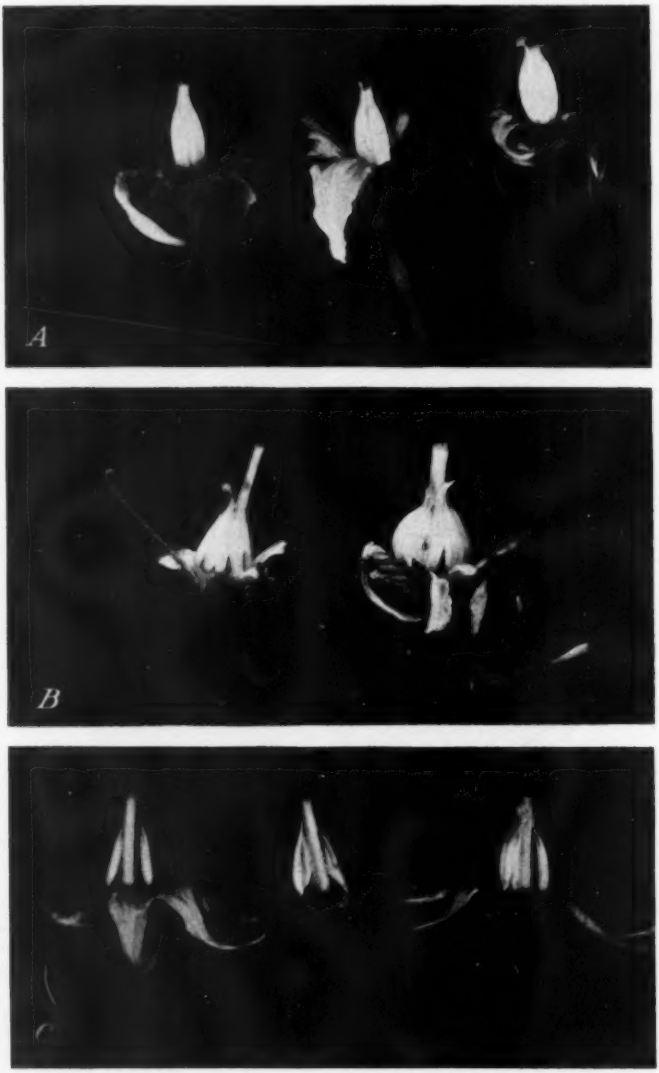

FIGURE 9.—Typical flowers of the second cluster of Marhio on April 11, 1935, (*A*) and of the second (*B*) and fourth (*C*) cluster of Ponderosa on April 11 and 24, 1935, respectively. Note the pronounced extent to which the relative length of the pistil and stamens changed from April 11 to April 24.

value for the pistil was 1.72 mm and for the stamens 0.30 mm for an increase of 1 mm in the pistil-stamens average length. This indicated the great extent to which change in length of the pistil was responsible for the distance the stigma was located above the tips of the stamens. In this respect the situation was comparable to that observed in Break O'Day, where the pistil was entirely enclosed within the staminal cone.

SERIES 4

Series 4 consisted of two lots of plants. The first lot of 22 varieties was composed largely of plants grown from seed sown March 9. These plants grew vigorously prior to potting on April 30 in manure-loam soil mixtures. The plants were grown both in pots and in a ground bed.

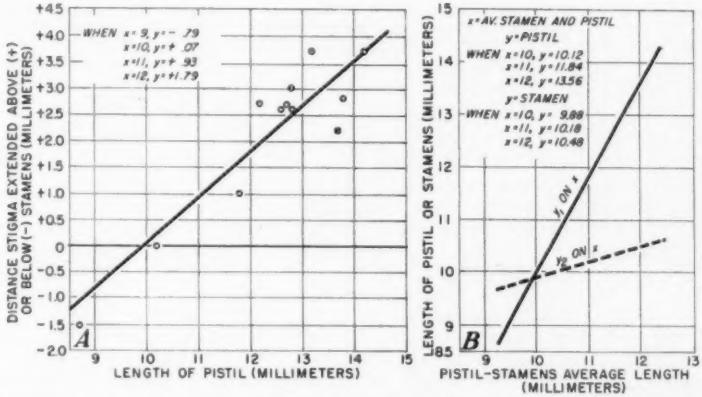

FIGURE 10.—*A*, The regression of pistil length minus stamens length (y) on length of pistil (x) in Ponderosa; $b = 0.86$ mm. *B*, The line y_1 on x shows the regression of pistil length on pistil-stamens average length (x), and the line y_2 on x the regression of length of stamens on the pistil-stamens average length for a plant of Ponderosa grown in sand; $b = 1.72$ and 0.30 mm, respectively.

The second lot of plants was grown from seed obtained from English and Scotch firms. The seed was sown April 13 and the plants were potted on April 18 in a manure-loam soil mixture.

The flowers of the first cluster of the first lot of plants were examined on May 5 when they were just reaching anthesis. On May 15 the flowers of the second and third clusters and on June 15 the flowers of the later-blooming clusters were examined to determine the relative length of the pistils and stamens. Similar examination of the plants of the second lot was made on June 15 and July 8.

A classification of the varieties of the first lot on the basis of the position of the stigma in relation to the tips of the stamens on May 5, May 15, and June 15 is given in table 4.

MAY 5 EXAMINATION

The examination of the flowers of the first cluster on May 5 showed that the varieties could be divided into four groups based on the position of the stigmas in relation to the tips of the stamens. Group

1 comprised those varieties in which the flowers had shorter pistils than stamens, group 2 those in which pistils and stamens were of equal length, group 3 those in which the pistils extended only slightly above the tips of the stamens, and group 4 those in which the pistils extended well above the tips of the stamens.

TABLE 4.—*Classification of American and English varieties of tomato on the basis of the position of the stigma in relation to the tips of the stamens*

FIRST LOT, APRIL TO JUNE 1936

May 5

Stigma well within staminal cone	Stigma slightly within staminal cone	Stigma flush with tip of staminal cone	Stigma slightly above tip of staminal cone	Stigma well above tip of staminal cone
No varieties in this class.	Dwarf Aristocrat. Marglobe. Michigan State Forcing. Potentate.[1] Pritchard.	Dwarf Champion. Gulf State Market. Marhio. Riverside Favorite.[1]	Globe. John Baer. Marvana. Veals.[1]	Beauty. Bonny Best. Chalk Early Jewel. Earliana. Early Stone. Greater Baltimore. Ideal. Lorillard. Marvel. Oxheart. Progress. Ponderosa.

May 15

No varieties in this class.	Dwarf Aristocrat. Dwarf Champion. Gulf State Market. Marglobe. Marhio. Michigan State Forcing. Potentate.[1] Pritchard. Riverside Favorite.[1]	Earliana. Globe. Ideal. Tuckswood.[1]	Beauty. Lorillard. Marvel. Progress. John Baer.	Bonny Best. Chalk Early Jewel. Early Stone. Greater Baltimore. Marvana Ponderosa.

June 15

Dwarf Aristocrat. Marhio. Michigan State Forcing. Potentate.[1] Pritchard.	Dwarf Champion. Earliana. Globe. Gulf State Market. Lorillard. Marglobe. Marvel. Veals.[1]	Beauty. Bonny Best. Early Stone. Greater Baltimore. John Baer. Marvana. Riverside Favorite.[1] Tuckswood.[1]	Chalk Early Jewel. Oxheart. Ponderosa.	

SECOND LOT, JUNE AND JULY 1936

June 15

Earliest of All.[1]	Best of All.[1] Essex Wonder.[1] Holyrood.[1] Perfection.[1]	Earliest.[1] Majestic.[1]		

July 8

Best of All.[1] Earliest of All.[1] Essex Wonder.[1] Holyrood.[1]	Earliest.[1] Majestic.[1] Perfection.[1]			

[1] Variety of English origin.

Table 4 includes a number of varieties which had not been previously examined. The flowers at anthesis on May 5 were the last of the first cluster and the first of the second. As might have been expected from previous observations, the pistils of the varieties Bonny Best, Earliana, Greater Baltimore, Lorillard, and Ponderosa extended well above the tips of the stamens. Included also in this group were the varieties Beauty, Chalk Early Jewel (fig. 11, *A*), Early Stone, Ideal, Marvel, and Oxheart. The pistils of Globe (fig. 11, *C*), Marvana, John Baer, and Veals were only slightly exserted. On the other hand, the stamens and pistils in Dwarf Champion, Gulf State Market, Marhio, and Riverside Favorite were of equal length. The varieties with pistils slightly inserted were Dwarf Aristocrat, Marglobe (fig. 12, *A*), Michigan State Forcing, Potentate, and Pritchard.

MAY 15 EXAMINATION

Examination of the flowers of the third cluster showed that the relative length of the pistils and stamens had changed in several varieties, the trend being toward a shorter pistil in relation to the stamens. The pistils of Bonny Best, Chalk Early Jewel, Early Stone, Greater Baltimore, Marvana, and Ponderosa were still considerably longer than the stamens, but in Earliana, Globe, Ideal, and Tuckswood the pistils and stamens were of equal length and in Dwarf Champion, Gulf State Market, Marhio, and Riverside Favorite they were slightly inserted. No varieties possessed pistils considerably shorter than the stamens at this time.

JUNE 15 EXAMINATION

On June 15 the pistils of all varieties were shorter in relation to the stamens than on May 15. Only the pistils of Chalk Early Jewel (fig. 11, *B*), Oxheart, and Ponderosa had exserted pistils. The pistils and stamens were now of equal length in Beauty, Bonny Best, Early Stone, Greater Baltimore, John Baer, Marvana, Riverside Favorite, and Tuckswood. On the other hand, the pistils were slightly inserted in Dwarf Champion, Earliana, Globe (fig. 11, *D*), Gulf State Market, Lorillard, and Marglobe (fig. 12, *B*). The pistils were much shorter than the stamens in Dwarf Aristocrat, Marhio, Michigan State Forcing, Potentate, and Pritchard.

Thus, during the period from May 5 to June 15 two important observations were made: (1) In all varieties there was a gradual reduction in the length of the pistil relative to that of the stamens, and (2) the different varieties varied in the amount of reduction that occurred. Certain varieties with pistils relatively short in relation to the stamens on May 5, as might be expected showed relatively little change during the period as compared to those varieties in which the pistils were considerably longer than the stamens on May 5.

Observations on Varieties of the Second Lot

JUNE 15 EXAMINATION

Of the varieties of English origin forming the second lot one group had pistils flush with the tips of the stamens and another group had pistils slightly shorter than the stamens. In the first group were Earliest (fig. 12, *C*) and Majestic (fig. 13, *A*) and in the second Best of

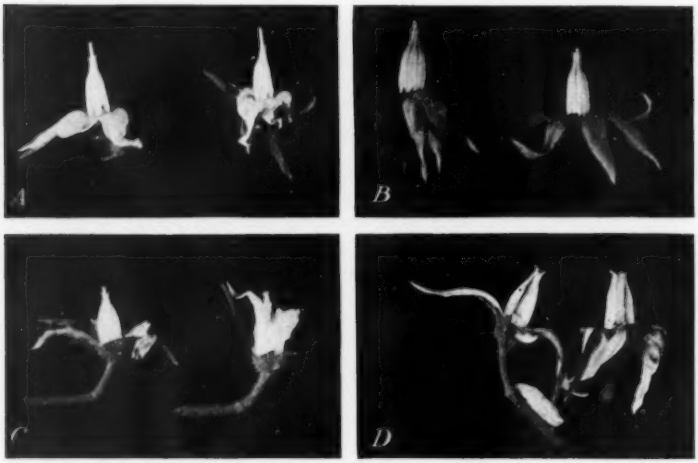

FIGURE 11.—Two flowers of the first cluster of Chalk Early Jewel on May 5, 1936 (*A*), and two from a later-blooming cluster on June 15, 1936 (*B*); note that the pistils were exserted at both times. A typical flower of Globe on May 5, 1936, is shown in *C*, with slightly exserted pistil, and in *D* two flowers of the same variety are shown on June 15 with pistils very slightly shorter than the stamens.

FIGURE 12.—Two flowers from the first cluster of Marglobe on May 5, 1936 (*A*), with pistils slightly inserted, and typical flowers from an upper cluster of the same variety on June 15, 1936 (*B*). Pistils in *B* are only slightly inserted and show very little change from *A*. Typical flowers of Earliest are shown on June 15 (*C*) and July 8, 1936 (*D*).

All, Essex Wonder, Holyrood, and Perfection. Earliest of All alone showed pistils well within the staminal cone.

JULY 8 EXAMINATION

The observations made of the succeeding clusters in these varieties showed that with the exception of two varieties, Earliest of All and Perfection, all showed a reduction in the length of the pistils relative to that of the stamens during the period from June 15 to July 8. The pistils of Earliest (fig. 12, *D*) and Majestic (fig. 13, *B*) were now slightly shorter than the stamens, whereas in Best of All, Essex Wonder, and Holyrood the pistils were well within the staminal cone; that is, considerably shorter than the stamens. It is interesting to note that even in those English varieties grown during the longest days of the year there was a change in relative length of the pistils and stamens. It should be kept in mind, however, that these plants were growing in a very vigorously vegetative condition at the time they were potted in the manure-loam mixture.

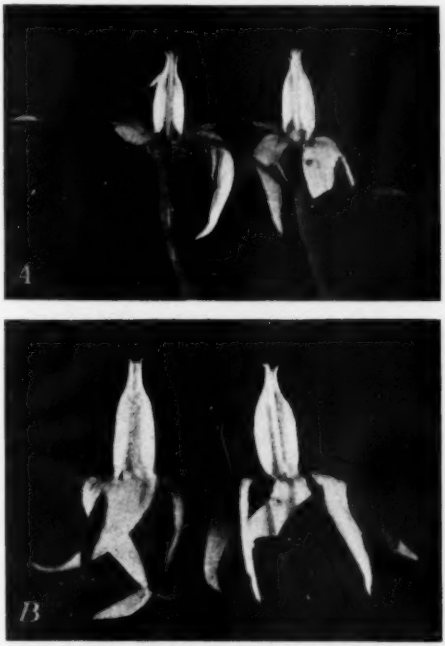

FIGURE 13.—Typical flowers of Majestic on June 15 (*A*) and on July 8, 1936 (*B*).

SERIES 5

Series 5 consisted of plants of 30 varieties grown in connection with another fruit-setting experiment. However, in view of the fact that the varieties showed marked differences in the relative length of the pistils and stamens in the flowers of the first cluster, the results of the observations are presented here.

The plants were grown from seed sown January 13, 1937. The seedlings were transplanted March 1 into a manure-loam mixture consisting of 1 part of manure to 12 parts of soil. A dilute solution of ammonium nitrate was added to each pot on March 8. The first flower of the first cluster reached anthesis on March 18.

The varieties are classified in table 5 on the basis of the relationship of the position of the stigma to the tips of the stamens. The value of this classification lies in the fact that the plants of the various varieties were grown during the same period in the same soil mixture and received the same treatment. Repetition of the experiment might give results different from those presented here, but in general the classification is very similar to that of the previous series.

TABLE 5.—*Classification of 30 varieties of tomato on the basis of the position of the stigma in relation to the tips of the stamens in flowers of the first cluster on March 18, 1937*

Stigma well within staminal cone	Stigma slightly within staminal cone	Stigma flush with tip of stamens	Stigma well above tips of stamens
No varieties in this class.	Best of All. Break O' Day. Earliest. Earliest of All. Grand Rapids Forcing. Marhio. Michigan State Forcing. Marglobe. Long Calyx. Glovel. Penn State. Pritchard. Rutgers. Holyrood.	Blair Forcing. Bonny Best. Globe. Comet. Perfection. Ideal. Tuckswood. Urbana. Lloyd Forcing. Sureset.	Indiana Baltimore. New Stone. Nystate. Norton. Ponderosa. Majestic.

In the first place, no variety had flowers of the first cluster with pistils considerably shorter than the stamens, that is, well within the staminal cone. Among the American varieties with the pistils slightly shorter than the stamens were Break O' Day, Marhio, Michigan State Forcing, Marglobe, and Pritchard. Those varieties with pistils and stamens of equal length included Bonny Best, Globe, and Ideal, and those with pistils considerably longer than the stamens included Indiana Baltimore (a selection of Greater Baltimore) New Stone, Norton, Nystate, and Ponderosa.

The varieties of English origin with pistils slightly within the staminal cone were Best of All, Earliest, Earliest of All, and Holyrood. The pistil and stamens of the varieties Comet and Perfection were of equal length, whereas the variety Majestic was characterized by flowers with pistils considerably longer than the stamens. This is not surprising in view of the observations made on the flowers of Majestic in series 4.

DISCUSSION

CHANGE IN RELATIVE LENGTH OF PISTIL AND STAMENS IN FLOWERS OF THE SAME VARIETY

The outstanding result of this study is evidence that the relative length of pistil and stamens varies in flowers of the same variety, as a result of the influence of environment, to a much greater extent than has previously been assumed. The observations of Burk (*6*) were confined to two varieties, Bonny Best and Princess of Wales. The pistil length of the first, a variety of American origin, was reported to be influenced by photoperiod and light intensity; whereas that of the second, a variety of the so-called English type, showed no effect of environment. In the present work all varieties studied, regardless of origin, showed some response to environment in respect to relative length of pistil and stamens. Considerable differences were noted between varieties in the degree to which they were affected by the same environment, but in each the relative length of the pistil and stamens was observed to vary from flower to flower within a single cluster, from cluster to cluster, and from plant to plant.

From the observations reported in this paper it is clear that the change in relative length of the two essential organs was due largely to variation in length of the female organ, but the stamens also were

concerned in the change (table 3). Casual inspection shows that there was a tendency for increasing length of pistil to be accompanied by increased length of stamens, but the regression lines in figures 4, 7, and 10, *B*, show that in all varieties examined the *b* value for pistil length was greater than the corresponding value for length of stamens. Thus it is apparent that the change in length of pistil was more important in the increase of the pistil-stamens average length than was length of stamens.

The data in table 3 indicate that the ovary as well as the style was concerned in the change in length of the pistil, although it is evident that the style was usually more important than the ovary in establishing the degree of pistil insertion or exsertion. Heretofore, it has been assumed that the style alone was concerned.

EXTENT OF THE VARIATION IN RELATIVE LENGTH OF PISTIL AND STAMENS

Despite the fact that the varieties studied were found to differ in the extent to which the relative length of the pistil and stamens was affected by environment, it would be hazardous to make comparisons between different varieties without measuring the length of the two organs. The variation between two varieties may appear greater than it is if one variety has both exserted and inserted pistils and the other usually only inserted pistils. For example, a variation within the variety Bonny Best which results in the change from a condition in which the pistil is exserted to one in which it is inserted (table 3) might appear greater than an equally pronounced change in Break O'Day whose pistils are usually enclosed within the staminal cones (table 3). The data given in table 3 for Break O'Day show values ranging up to 3 mm in the extent to which the pistils were shorter than the stamens. In view of the fact that measurements of the length of the two organs were made in only nine varieties, the writer hesitates to classify the other varieties on the basis of the relative extent to which the two organs were affected by the environmental factors involved in this study. However, it should be noted that the relative length of the pistil and stamens in both the varieties of American and English origin was definitely influenced by environment. As a group the American varieties seemed to show a more pronounced change, but in a few English varieties the change was as great. The English variety Majestic is an example and, as the literature indicates, Comet also frequently shows a pistil relatively long in relation to the stamens. On the other hand, it is probable that not all varieties of American origin show an equally pronounced change in the relative length of the pistil and stamens. This, however, cannot be definitely stated without measurements of the two organs such as were carried out with the nine varieties. In this connection it is interesting to note that Marglobe, Michigan State Forcing, and Pritchard tend to have the pistils enclosed well within the staminal cones in the greater proportion of flowers. Only infrequently has the writer observed flowers of these varieties with pistils exserted.

CLASSIFICATION OF VARIETIES ON THE BASIS OF PISTIL EXSERTION OR INSERTION

The change in relative length of the pistil and stamens within a variety may result in a change from a condition in which the pistil is exserted to one in which it is inserted. Obviously the relative length

of the two organs in a variety is also governed by its genetic constitution, and the relative length of the two organs at any one time is the result of the interaction of heredity and environment. In a variety such as Ponderosa, in which there is a natural tendency toward a pistil long in relation to the stamens, the effect of environment would necessarily be extremely pronounced if flowers were to occur with pistils shorter than the stamens. In the experiments reported herein the extent of the change in Ponderosa was little if any greater than in several other varieties such as Globe, Bonny Best, and Greater Baltimore, and in consequence a flower of Ponderosa with a pistil shorter than the stamens was rarely noted.

On the other hand, in a number of varieties, such as Blair Forcing, Bonny Best, Earliana, Globe, and Lorillard, a change in relative length of the pistil and stamens similar to that occurring in Ponderosa resulted in a change from a condition in which the pistils were exserted to one in which they were inserted. Finally, in a variety such as Break O'Day, which has inherited a genetic factor or factors for a short pistil in relation to stamens, a variation up to 3 mm, practically equal to that occurring in the above-mentioned varieties, took place without resulting in flowers with pistils exserted.

Despite the fact that a classification of varieties based on this tendency to have pistils exserted, inserted, or both, may be too arbitrarily interpreted, a rough classification of a number of the varieties used in this study has been made. Some varieties observed have not been classified because of insufficient evidence. It should be borne in mind that any or all of the varieties placed in one group may infrequently develop flowers with either pistils inserted or exserted, or both.

The varieties whose flowers usually have pistils longer than stamens are Chalk's Early Jewel, Oxheart, and Ponderosa.

The varieties whose flowers vary from exserted pistils to inserted pistils are Beauty, Blair Forcing, Bonny Best, Earliana, Globe, Greater Baltimore, Ideal, John Baer, Lorillard, Majestic, Marhio, Marvel, Riverside Favorite, and Veals.

The varieties whose flowers usually have pistils inserted are Best of All, Break O'Day, Comet, Dwarf Aristocrat, Earliest, Earliest of All, Essex Wonder, Grand Rapids Forcing, Gulf State Market, Holyrood, Marglobe, Michigan State Forcing, Perfection, Potentate, and Pritchard.

The majority of the varieties making up the last group are of English origin. Those of English origin are frequently characterized by a greater number of flowers to a cluster and smaller fruits than usually occur on the varieties cultivated in the United States. However, Break O'Day, Michigan State Forcing, and Pritchard are definitely of American origin, although in the greater number of instances they have originated through hybridization of American and European varieties.

ENVIRONMENTAL CONDITIONS ASSOCIATED WITH CHANGE IN RELATIVE LENGTH OF PISTIL AND STAMENS

In the experiments described above the maximum length of pistil in relation to stamens was obtained with plants that were developing the first or second flower clusters during February (fig. 14) or early March. These plants were growing during a period of relatively

short daylight, with light of low intensity, and with an abundance of readily available nitrogen in the sand or soil media. The plants were representative of those grown for the spring greenhouse crop. Flowers with long pistils in relation to the stamens have also been frequently obtained during November and December in the upper clusters of plants bearing a considerable number of fruits on the lower clusters. In the first instance (February) the daylight period was gradually lengthening; in the latter period it was gradually shortening. In both instances the particular portion of the plant in the region of the developing flower cluster would be considered soft, succulent, and not vigorously vegetative. (Vigorously vegetative is assumed to mean a rapid rate of growth associated with a large diameter of stem relative to length.) In both instances the supply of readily available

FIGURE 14.—Flowers of the first cluster of Marhio on February 13, 1935. Note the extreme length of the pistils.

nitrogen is high. The stem and leaves are light green rather than the dark green associated with a vigorously vegetative condition.

A moderate elongation of the pistil is but a transition stage from the long to the short pistil. It is separated from the extreme of each only in degree. A moderate elongation occurs in the second, third, and even fourth cluster of the same plants that show a long pistil in February. With each succeeding flower or flower cluster the pistil frequently is shorter in relation to the stamens. Such a condition develops during late winter and early spring as the days lengthen and the light is of greater intensity. A moderate elongation also occurs on plants growing under an environment which produces a vigorously vegetative type of growth, thick stems, large, dark-green leaves, and rapid growth extension. As indicated by the flowers of the first and second cluster of the vigorously vegetative plants of series 1 grown in London, a moderate elongation of the pistils in Marhio may have pronounced effects upon the set of fruit when the pistils and stamens are of equal length. Such a situation prevents adequate self-pollination. The principal difference in the environment responsible for long pistils as opposed to those of moderate length is a shorter period of light or light of lower intensity, an abundance of available nitrogen being present in both instances.

Finally the short pistil in relation to the stamens is obtained in the upper clusters of the same plants that showed moderate elongation of the pistils in the second, third, and fourth clusters. It is separated only in degree from the moderately long and the long pistils. From the short to the long pistil within a variety, a series of flowers may be developed, each with its pistil slightly longer than that of the preceding. The greater proportion of flowers on plants grown under long days with light of high intensity and only a moderate amount of readily available nitrogen tend to have short pistils in relation to the stamens. Plants growing in the field or in sand culture in midsummer and not forced with a large supply of available nitrogen usually show this relationship. In fact, it might be predicted that plants with the shortest pistils in relation to the stamens would be those bordering on a slight nitrogen deficiency. This in itself is rather significant, since with the tomato nitrogen deficiency has a more pronounced effect upon the development of the pistil than upon the stamens (until the deficiency becomes extreme).

Burk (*6*) stated that the lengthened pistil in relation to the stamens in Bonny Best was associated with light of low intensity and was also related to photoperiod. The experiments reported herein confirm his conclusion in regard to light intensity. Photoperiodism as used originally was applied to the effect of light periods upon the differentiation of floral primordia, and in this sense the tomato must be regarded as insensitive to photoperiod since its floral primordia are differentiated over a very wide range of light periods, from those of 4 or 5 hours duration to 24 hours of continuous illumination. Arthur, Guthrie, and Newell (*1*) showed that even fruits will develop in 7 hours of artificial illumination if the light intensity is sufficiently high. To be sure, flowers formed under a very short period of light may not attain the anthesis stage. Whether they will reach anthesis under such a light period depends, so far as is known at present, upon the suitability of the light for a rapid rate of photosynthesis.

For several years the writer has been carrying on supplemental illumination experiments with tomato plants grown from November to January. The additional illumination has resulted in bringing to anthesis flowers which on the plants with no supplemental illumination would have abscised while still small. In these experiments the flowers on the plants grown with supplemental illumination from 4 p. m. to 12 p. m. were intermediate in size between those that were grown without supplemental illumination and those illuminated from 9 a. m. to 12 p. m. The greater proportion of the flowers on the plants suffering from severe deficiency of carbohydrate did not even reach anthesis. Photoperiod in the tomato, so far as it is concerned with the change in flower structure considered in this paper, appears to be largely a matter of the effect of light intensity upon the process of photosynthesis.

PHYSIOLOGICAL CONDITIONS ASSOCIATED WITH CHANGE IN RELATIVE LENGTH OF PISTIL AND STAMENS

The nature of the environmental factors just described permits an understanding of the physiological conditions which are responsible for or are associated with the change in the relative length of the pistil and stamens. Microchemical analyses of the plants used in this study were not made, but indirect evidence concerning their

chemical composition can be obtained from analyses made by two investigators who have produced plants of similar growth status as a response to a similar environment.

It should be recalled first that the tomato plants which showed the maximum length of pistil in relation to stamens were fairly light green in color, soft, and succulent, but not vigorously vegetative. Since the grouping used by Kraus and Kraybill (*13*) which classifies plants on the basis of their nitrogen-carbohydrate relationship is already familiar, it will be used for classifying the plants observed in this study. In the writer's opinion, the plants with pistils long in relation to stamens would fall in class 1, on the border line of the vigorously vegetative plants placed in class 2. It is now evident that in reality class 1 comprises plants that are carbohydrate-deficient. Nightingale, Schermerhorn, and Robbins (*15*) analyzed plants representative of these classes. The growth status of the plants used by the writer which had the long pistils in relation to stamens indicate that they would undoubtedly fall between the weakly vegetative plants of series B and the vigorously vegetative plants of series A in the experiments of Nightingale, Schermerhorn, and Robbins. The data for the reserve carbohydrates in the plants of series B (class 1) substantiate the conclusion that carbohydrate deficiency, in reality, characterizes the light green, soft, succulent tomato plant. Evidence in support of this conclusion is available from the results (unpublished) of the writer's supplementary illumination experiments with the tomato plants showing this type of growth.

The rank-growing, vigorously vegetative tomato plant may be taken as an example of a growth condition which is associated with an intermediate length of pistil in relation to the stamens (plants of series 1 in this study). Kraus and Kraybill (*13*), and Nightingale, Schermerhorn, and Robbins (*15*), have given the chemical composition of such plants. The data for series Q of Kraus and Kraybill and for series A of Nightingale, Schermerhorn, and Robbins are pertinent in this connection. In the upper stems of the plants of series Q, in the vicinity of the developing flower clusters, the starch content was 1.06 percent and the sucrose 0.20 percent of the dry weight. This obviously indicates little or no carbohydrate reserve, a condition which appears to be characteristic of the vigorously vegetative type of plant. Miller, *14, p. 544* stated the same conclusion when he said that in vigorously vegetative plants "there appears to be an available supply of both carbohydrates and nitrogen and the balance between them is such as to produce the best vegetative growth without leaving a residue of carbohydrate." The last phrase indicates that Miller had concluded that vigorously vegetative plants had sufficient carbohydrate material for vegetative growth but little or no residue after the demands of the stem and leaves had been satisfied. A rapid rate of formation of new cells together with a subsequently rapid rate of amino acid synthesis (associated with an abundance of readily available nitrogen) does not insure that sufficient carbohydrates will be available for the development of functional sex organs and gametes. That sufficient carbohydrate material is formed in photosynthesis in such plants for maintaining a vigorously vegetative growth (thick stems in relation to their length) of stems and leaves is self-evident, but that this necessarily means a sufficient supply remaining for the production of flowers with functional sex organs and gametes has never been demonstrated. In fact,

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unpublished data gathered by the writer indicate that the failure of the flowers of the vigorously vegetative plant to set fruit is due to two causes, either one or both of which may be responsible in any given situation for the failure of fruit development. The first is the production of pollen with little or no fertility (*10*) and the second the development of flowers with a pistil at least intermediate in length between the short and the long condition. It is true, however, that an intermediate length of pistil may develop while a considerable proportion of the pollen is still fertile, a fact which indicates great sensitivity of the pistil to a low amount of reserve carbohydrate. Yet, the evidence at present available points to the conclusion that a mild deficiency of carbohydrate in the developing flower results in a lengthening of the pistil in relation to the stamens. Other factors, such as phytohormones, are not necessarily ruled out. It is possible that the mild deficiency of carbohydrates or the failure of deposition of carbohydrate material in the cell walls of the developing flowers permits a given amount of hormone to induce a greater cell extensibility or even greater cell division in the pistil than in the stamens, which would thus account for the change in the relative length of the two organs.

SIGNIFICANCE OF THE MORE PRONOUNCED CHANGE IN LENGTH OF PISTIL THAN OF STAMENS

Change in length of pistil, which is morphologically considered as a modified leaf, may be akin to similar elongation of the true leaves occurring on the same plant under the same environmental conditions. That the length of the stamens does not usually change proportionately does not vitiate this explanation, since during the course of the phylogenetic development the stamens have undergone a somewhat different modification from that which occurred in the development of the pistil. In fact, it would appear that these organs have a different metabolic gradient which accounts for the more pronounced change in the pistil than in the stamens under the same environment. That differences exist in the physiological mechanism of the developing male and female sex organs is suggested by Riddle (*16*) and Sansome (*17*) and is indicated by the work of Satina and Blakeslee (*18*), Bouillene, Bouillene, and Ghenne (*2*), Joyet-Lavergne (*12*), and Talley (*22*). In view of this it might be expected that any given environmental factor, such as light of moderate intensity with accompanying high inorganic nitrogen, would have a more pronounced effect upon the spatial arrangement of one sex organ than upon the other, a situation which occurred in the experiments reported herein.

RELATION OF THE DATA PRESENTED TO THAT OF OTHER INVESTIGATORS

Failure of workers to take cognizance of the pronounced effect of environment upon the relative length of pistil and stamens in different varieties of *Lycopersicum esculentum* has resulted in descriptions which appear to be contradictory but which can be shown to fit into a harmonious whole if certain additional facts are considered.

Harley (*9*) mentioned the pollination on March 1 of protruding pistils of the Lorillard variety. This condition—pistils longer than stamens—might be expected in early March, but, as indicated in table 3, the pistils in later-formed flowers would be within the staminal cones. Bouquet (*3*) presented a photograph of flowers of the Earliana

variety with pistils shorter than stamens but later (*4*) he described the pistil of the tomato flower as "the erect central organ of the flowers protruding through the stamens as they come together at the tip." Swingle (*21*) described the Stone variety as possessing a pistil which extended one-sixteenth of an inch beyond the stamens, whereas in the variety Grand Rapids Forcing he found the pistil "about hidden beneath the whorl of stamens." Crane (*8*) in England, who was the first investigator to describe specifically the characteristic aspects of pistil and stamen length, declared that in the usual variety of *Lycopersicum esculentum* the stigma appears slightly below the apex of the stamens. However, he did report the discovery of two abscising flowers in the open with stigmas protruding about one-eighth of an inch above the stamens. Crane's varieties were those commonly grown in England, but the data in tables 4 and 5 show that even in varieties of English origin, some of which produce many small-sized fruits to a cluster, a change in the relative length of the pistils and stamens is common in flowers on the same plant.

Tracy (*23*), working with American varieties, inferred that the characteristic flower involved an elongating style which grew from within the staminal cone to beyond the tips of the stamens, often before the pollen matured. However, the diagram in his textbook, reportedly drawn from a photograph, shows the flowers at anthesis with pistils enclosed within the staminal cone. White (*24*) recognized that varieties differ in the extent of pistil exsertion. He classified 10 varieties in two groups, one group consisting of those with pistils extending beyond the tips of the stamens and the other with pistils shorter than the stamens. Among the varieties in the first group was Comet and among those in the second were Hubert Marvel and Stirling Castle. Schneck (*19*) considered the flower with the portruding pistil to be but a later development of a flower with the pistil enclosed, and presented such a diagram of a typical flower. He showed two photographs of Globe, one with the pistil enclosed within the staminal cone and the other with the pistil protruding slightly. He described the varieties Bonny Best and John Baer as possessing pistils and stamens of equal length at the beginning of pistil receptivity even though the pistils of Bonny Best might show considerable elongation thereafter. He stated that the variety Beauty had pistils considerably longer than the stamens. Schneck concluded, however, that the flowers of all American varieties are not similar to those of Globe. Furthermore, in contrast to White's description of Comet, he found that the variety had a pistil much shorter than the stamens, a condition reported to be similar to that occurring in other varieties of the so-called English type. He stated that in such varieties as Grand Rapids Forcing and Lord Roberts the pistils were considerably shorter than the stamens at the time of pollination. Schneck's observations were made "during late winter and early spring," a period when a marked difference in the relative length of the pistil and stamens occurs in flowers of the same and succeeding clusters. The contradictory observations of White (*24*) and Schneck concerning the Comet variety can be reconciled. Under the usual summer environment, when days are long and opportunities for photosynthesis are adequate, this variety has the pistil enclosed within the staminal cone. It appears very likely that the plants with protruding pistils, observed by White, were grown under conditions producing a more vegetative type of growth or under lower

light conditions than the plants of the same variety grown by Schneck in which the pistils were shorter than the stamens.

Jones and Rosa (*11*) present an illustration of two so-called types of flowers in *Lycopersicum esculentum*, one short-styled with stigma enclosed within the staminal cone and the other long-styled with the style projecting beyond the tips of the stamens. But the implication involved in this short-styled type is dismissed with the following statement:

> During maturation of the flower the pistil elongates rapidly, pushing the stigma beyond the staminal cone, which insures selfing, if pollen is being shed. In some varieties, however, and probably in some flowers of all varieties, the style elongates before the anthers dehisce, thus exposing the stigma to foreign pollen.

Thus Jones and Rosa conclude that the characteristic flower structure of all the so-called American varieties at least, involves a style which extends beyond the tips of the stamens.

The two flower types shown by Jones and Rosa are such as might be obtained on the same plant or from two plants grown at different seasons of the year. The so-called long-styled might be found on the first cluster of the Globe variety grown in March and the second type, on three or four later clusters of the same plant. Cooper (*7*) in 1927 in describing the tomato flower stated that the stigma "extends slightly beyond the apex of the androecium." He showed a photograph of a flower, however, with the pistil and stamens of equal length.

Smith (*20*) stated that the variety Bonny Best has a "comparatively long style," and that "during the maturation of the flower the pistil elongates rapidly, pushing the stigma through the staminal cone." Bouquet (*5*) in 1932 presented measurements of the pistil length of several varieties indicating that the differences in pistil length of some varieties were significant statistically. He stated that the varieties of the English type, such as Best of All, Kondine Red (Tuckswood), Comet, Princess of Wales, Grand Rapids Forcing, and Crackerjack, had styles located 1 mm below the apex of the stamens; whereas other varieties, such as Globe and Bonny Best, had styles flush with or above the tips of the stamens. It is thus to be noted that the idea is prevalent that the pistil elongates during anthesis (possibly just before anther dehiscence) in the flowers of the varieties that are characterized by pistil exsertion. It has not been stated by the present writer that lengthening of the pistil never occurs after the flowers reach anthesis. Measurements of the pistil of the same flower during the course of these experiments indicate that a slight elongation may be an infrequent occurrence, but when conditions are favorable for pollination and fertilization very rapid enlargement of the ovary occurs before the anthers have withered. Such enlargement pushes the style to a position higher in relation to the anthers than occurred in the same flower during the period immediately before fertilization. Failure to note that the fruit has "set" may lead one to assume that the flower structure has been changed when, as a matter of fact, up to the time of fertilization there was no change whatever in the relative length of the pistil and the stamens.

In spite of the contradictory statements in the literature cited, there is a fairly general agreement that the common American-grown varieties characteristically possess flowers with pistils extending beyond the tips of the stamens while those of English origin generally show no pistil exsertion.

SUMMARY AND CONCLUSIONS

The most important result obtained from this study of the flower structure of tomato is evidence that the relative length of the pistil and stamens in flowers of the same variety is influenced by the environment to a much greater extent than has previously been assumed.

All varieties studied, regardless of origin, showed a definite response to environment in respect to the relative length of pistil and stamens.

The change in relative length of the two essential organs was largely due to variation in length of the pistil. The length of stamens varied also but to a lesser extent.

The ovary as well as the style was concerned in the change in length of the pistil. Usually, however, the style was the more important in establishing the degree of pistil exsertion or insertion.

Within each group of varieties, both of American and English origin, differences occurred in the extent or degree to which the relative length of the pistil and stamens varied as a response to environment.

In certain varieties the fact that the pistil was enclosed within the staminal cone masked the degree of change induced by the environment. This was true of the variety Break O'Day in which the pistils were shorter than the stamens by variations up to 3 mm. This variation was no less than that shown by Ponderosa, but in the case of Ponderosa the variation was easily apparent since the pistils extended above the stamens The change was no greater in such a variety as Globe in which the pistils varied from a position longer than the stamens to one correspondingly shorter.

A classification of a number of varieties is presented on the basis of the relation of the length of the pistils to that of the stamens. One group includes the varieties having pistils longer than the stamens, another those having pistils shorter than the stamens, and a third those in which the pistils varied from a position longer to one shorter than the stamens.

The maximum length of pistil in relation to the stamens was obtained when the plants were growing during a period of relatively short daylight, under light of low intensity, and with an abundance of readily available nitrogen. A moderate elongation of the pistil was but a transition stage from the long to the short pistil. A moderately long pistil was produced when the light period was slightly longer or the light was of higher intensity than that which favored the development of the long pistil. The short pistil in relation to the stamens was produced when the days were long, the light of high intensity, and the supply of readily available nitrogen only moderate. An increase in length of the pistils in relation to the stamens appears to be associated with if not caused by carbohydrate deficiency. The degree of change from the short to the long pistil seems to be positively correlated with the degree of deficiency.

It seems probable that the difference in the degree of response of the two sex organs to the same environmental influence is due to differences in the physiological mechanism of the male and female organs.

In the light of the results obtained it is apparent that the conflicting statements in the literature concerning the relative length of the pistil and stamens in different varieties are due in large part to the fact that the plants described were grown at different seasons of the year and under different environmental conditions, particularly as regards available light and nitrogen supply.

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A MOSAIC DISEASE OF RADISH IN CALIFORNIA[1]

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INTRODUCTION

A mosaic disease of the cultivated radish (*Raphanus sativus* L.) was prevalent in market gardens near San Pablo, in the San Francisco Bay section of California, during the winter months of 1933–34 and has been observed in subsequent years. Although infection was widespread, little or no damage to the plants resulted. The wild or escaped radish, a common weed along ditchbanks and waste places, according to Jepson (*6*),[3] was also found to be highly susceptible and probably serves as an important reservoir for the virus in this section. In 1936, J. H. Freitag of the Division of Entomology and Parasitology, University of California, observed the disease on cultivated radish near Colma.

The results of studies on transmission, host range, and properties of the radish mosaic virus are presented in this paper.

REVIEW OF LITERATURE

A mosaic disease of radish was found in Indiana by Gardner (*3*) in 1925.

Ogilvie (*8*) observed that wild and garden radishes (*Raphanus raphanistrum* L. and *R. sativus*) were affected by a mosaic disease in Bermuda which caused distortion and frequently blisterlike areas of the leaves.

In 1933, Hino (*5*) reported a mosaic disease of radish on the cultivated variety Daikon in Japan.

Additional reports of virus diseases occurring on other closely related species were found, mention of which may be of interest. In 1924 Kulkarni (*7*) described a mosaic disease of the cultivated rattail or serpent radish (*Raphanus sativus* L. var. *caudatus* Alef.), known in India as Mogri. This disease, observed at Poona in 1922, caused mottling on the leaves, stems, and pods during the early stages of infection. Later symptoms consisted of blanching, blistering, and distortion of the leaves and pods, checking of flower and fruit formation, and stunting of the plants.

In 1932, Fukushi (*2*) listed a mosaic disease of *Raphanus macropoda* Lév. which he accredited to Takimoto (*10*).

A virus disease of jointed or white charlock (*Raphanus raphanistrum* L.) was reported from South Africa in 1931 by Van der Byl (*16*). The virus which attacked this annual weed, known as ramenas, was also found to infect turnips. Hino (*5*) also reported infection on this species in Japan, known there as Hama-daikon.

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[1] Received for publication June 20, 1938.

[2] The writer is indebted to Dr. M. W. Gardner for advice and assistance and to Prof. B. A. Madson and W. W. Mackie for providing greenhouse space and facilities. The assistance of nontechnical employees of the Federal Works Progress Administration is acknowledged.

[3] Italic numbers in parentheses refer to Literature Cited, p. 129.

A mosaic disease of Daikon (*Raphanus* sp.) has recently been reported from Hawaii (4).

SYMPTOMS OF THE DISEASE

The symptoms produced by the radish mosaic virus discussed in this paper were identical in the field and greenhouse. Under greenhouse conditions, at temperatures ranging from 13° to 19° C., the first symptoms consist of small, roughly circular to irregular chlorotic lesions which occur indiscriminately between and adjacent to the veins (fig. 1, *A*). Fusion of some of these lesions is not uncommon (fig. 1, *A*). Within a few days, the chlorotic lesions become more numerous (fig. 1, *B*) and soon replace the normal, dark-green tissue, imparting a distinctly chlorotic color and a coarse mottle (fig. 1, *C*), in contrast to the normal, healthy condition (fig. 1, *D*). After 10 days to 2 weeks, the normal, dark-green tissue appears as irregularly-shaped, nonraised islands on a conspicuous, yellowish-green, chlorotic background (fig. 2). There is little or no leaf distortion, although occasionally raised, dark-green islands have been observed on radish plants in the greenhouse within a month after inoculation. Necrotic lesions and stunting of infected plants are not known to occur either in the field or in the greenhouse.

MATERIALS AND METHODS

The radish mosaic virus which was used in these studies came from a naturally infected white radish plant grown near San Pablo, Calif. By mechanical inoculation, the virus was transferred to healthy radish seedlings in the greenhouse. Subsequent monthly transfers of the virus have been made since 1934. White Icicle radish seedlings were selected as the standard test plant for recovery of the virus from infected plants and for property studies.

All inoculations were made in a greenhouse where temperatures ranged from 13° to 19° C. The methods followed were essentially those described in a recent paper (*12*). Mechanical inoculations were made by dusting the leaves with 600-mesh, powdered carborundum (*9*), and lightly rubbing with absorbent cotton dipped in juice from a diseased plant.

TRANSMISSION

The radish mosaic virus was readily transferred by rubbing. The use of carborundum (*9*) did not increase the percentage of transmission. As a result of numerous tests, the incubation period was determined as ranging from 9 to 18 days.

Studies on insect transmission of this disease involved the use of the cabbage aphid (*Brevicoryne brassicae* (L.)), the false cabbage or turnip aphid (*Lipaphis pseudobrassicae* (Davis)), and the green peach aphid (*Myzus persicae* (Sulzer)), all of which, according to Essig (*1*), breed naturally on radish and other cultivated and wild crucifers. Numerous, unsuccessful attempts were made to transmit the virus by means of these three species of aphids.

Tests for seed transmission were limited to seed obtained from 12 diseased radish plants which had previously been inoculated by mechanical means in the greenhouse. Seeds were planted in a flat of autoclaved soil. After 30 days, 757 plants were examined, all of

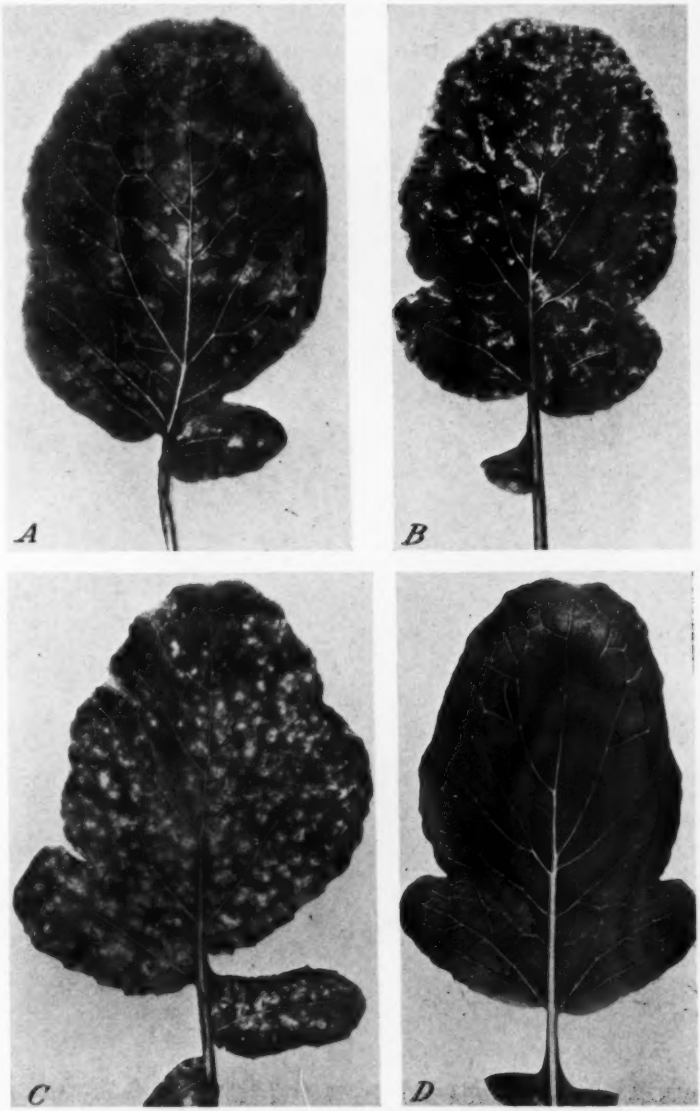

FIGURE 1.—Symptoms produced by the radish mosaic virus on White Icicle radish leaves after mechanical inoculation in the greenhouse at 13° to 19° C.: *A*, Early symptoms, consisting of small, circular to irregular, chlorotic lesions scattered indiscriminately between and adjacent to the veins; *B* and *C*, intermediate symptoms, consisting of irregularly-shaped light- and dark-green areas which collectively produce a coarse mottling; *D*, noninoculated control.

which were healthy, indicating that the virus is probably not seed-borne.

EXPERIMENTAL HOST RANGE

The following varieties of radish were tested in the greenhouse by mechanical inoculation and were found to be highly susceptible:

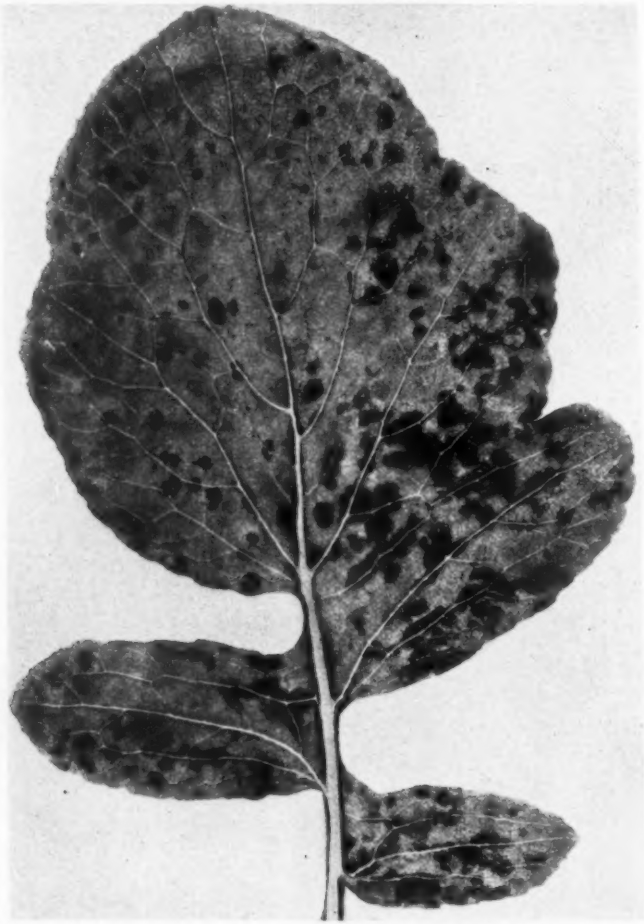

FIGURE 2.—Late symptoms produced by the radish mosaic virus on White Icicle radish leaf after mechanical inoculation in the greenhouse at 13° to 19° C. The normal, dark-green tissue appears as irregularly-shaped, nonraised islands on a conspicuous, yellowish-green, chlorotic background.

Cincinnati Market, Crimson Giant Forcing, Early Scarlet Globe Medium Top, Early Scarlet Globe Short Top, Early White Giant Summer or Stuttgart, French Breakfast, Half Long Deep Scarlet, Half Long Black Winter, Long Black Spanish, Long Scarlet, Long

White Japanese, Round Black Spanish, Scarlet Turnip, Scarlet Turnip White Tip, Sparkler, White Icicle, and White Strasburg. No resistant varieties have been found.

By means of mechanical inoculation, the radish mosaic virus was transmitted to 19 species of plants representing 9 genera in 4 families (table 1). The symptoms produced by mechanical inoculation on some cruciferous hosts are shown in figures 3 and 4; on species of Nicotiana in figure 5. The virus was recovered from all infected plants except spinach.

TABLE 1.—*Plants susceptible to the radish mosaic virus, as indicated by mechanical inoculation of greenhouse-grown seedlings, and symptoms characteristic of infection*

Family	Species and common name	Symptoms produced
Cruciferae	*Brassica oleracea* L. var. *acephala* DC. (kale).	Systemic infection. Diffuse chlorotic lesions on leaves.
	B. oleracea var. *gemmifera* DC. (Brussels sprouts).	Do.
	B. oleracea var. *botrytis* L. (cauliflower-var. February; sprouting broccoli-var. Italian Green Sprouting).	Do.
	B. oleracea var. *capitata* L. (cabbage-var. Winter Colma).	Systemic infection. Chlorotic and necrotic lesions on leaves.
	B. oleracea var. *caulorapa* DC. (kohlrabi)	Systemic infection. Mottling.
	B. nigra (L.) Koch (black mustard)	Do.
	B. alba (L.) Boiss. (white mustard)	Do.
	B. arvensis (L.) Ktze. (charlock)	Do.
	Capsella bursa-pastoris (L.) Medic. (shepherds-purse).	Do.
	B. pe-tsai Bailey (pe-tsai)	Local infection. Chlorotic followed by necrotic lesions and vein necrosis on inoculated leaves only.
	B. juncea (L.) Coss. (Chinese or leaf mustard).	Systemic infection. Mottling, midrib curvature and rugosity with downward curling of leaves.
	B. rapa L. (turnip-var. Purple Top White Globe).	Systemic infection. Mottling and severe distortion of leaves.
	Matthiola bicornis DC. (evening scented stock).	Systemic infection. Mottling, followed by death of plants.
	Malcomia maritima R. Br. (Virginian stock).	Systemic infection. Necrotic streaking of petioles and necrotic lesions on leaves, followed by death of the plants.
	Raphanus sativus L. var. longipinnatus Bailey (Chinese radish).	Systemic infection. Vein clearing, mottling, with bleaching of leaves in late stages of infection.
	B. adpressa Boiss.	Systemic infection. Chlorotic halos with green centers. Lesions averaged 3/16 of an inch in diameter.
Chenopodiaceae	*Chenopodium album* L. (lambsquarters or white pigweed).	Local infection. Light-tan, necrotic lesions on inoculated leaves only.
	C. murale L. (sowbane or nettle-leaf goosefoot).	Do.
	Spinacia oleracea L. (spinach-var. Boomsdale).	Systemic infection. Yellow lesions which later tend to fuse. Young leaves show midrib curvature. Marked leaf distortion, dwarfing, and occasional death of plants.
Ranunculaceae	*Delphinium ajacis* L. (rocket larkspur)	Systemic infection. Necrotic lesions on leaves and necrotic streaks on petioles.
Solanaceae	*Nicotiana glutinosa* L.	Local infection. Faint, chlorotic lesions on inoculated leaves only.
	N. tabacum L. (tobacco-var. Turkish)	Do.
	N. tabacum L. (tobacco-var. White Burley)	Systemic infection. Chlorotic lesions.
	N. rustica L. var. *humulis* Schrank	Do.
	N. langsdorffii Weinm.	Systemic infection. Chlorotic lesions with occasional necrotic lesions surrounded by yellow halos.

FIGURE 3.—Local infection produced after 32 days on Chinese cabbage (Pe-tsai) by mechanical inoculation with the radish mosaic virus in the greenhouse at 13° to 19° C. Necrotic lesions with centers of green tissue appeared only on inoculated leaves.

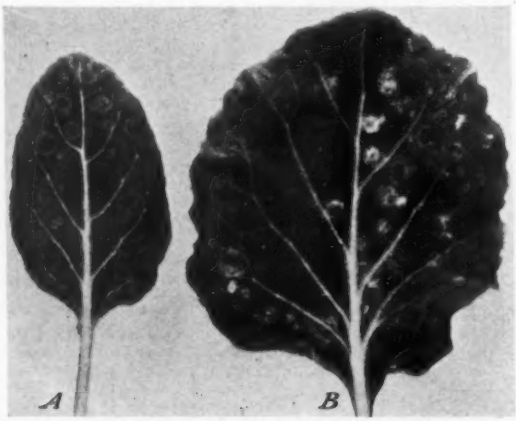

FIGURE 4.—Systemic infection produced by mechanical inoculation with the radish mosaic virus in the greenhouse at 13° to 19° C.: *A*, Chlorotic lesions on February cauliflower leaf, 16 days after inoculation; *B*, necrotic lesions with centers of green tissue on Winter Colma cabbage leaf, 22 days after inoculation.

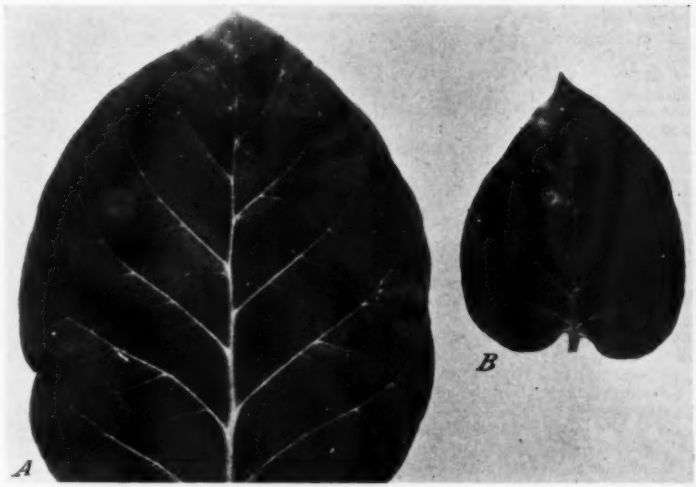

FIGURE 5.—Local infection produced after 21 days by mechanical inoculation with the radish mosaic virus in the greenhouse at 13° to 19° C.: *A*, Large, local, chlorotic lesions on inoculated leaf of Turkish tobacco; *B*, small, local, chlorotic lesions on leaf of *Nicotiana glutinosa*.

No infection was obtained by mechanical inoculation in 52 species of plants representing 45 genera in 25 families, as follows:

Begoniaceae:
- Fibrous-rooted begonia (*Begonia semperflorens* Link and Otto)

Boraginaceae:
- Forget-me-not (*Myosotis alpestris* Schmidt)
- Common heliotrope (*Heliotropium peruvianum* L.)

Campanulaceae:
- Canterbury-bells (*Campanula medium* L.)

Caryophyllaceae:
- Sweet-william (*Dianthus barbatus* L.)
- Babysbreath (*Gypsophila paniculata* L.)

Compositae:
- Head lettuce (*Lactuca sativa* L. var. *capitata* Hort.) var. Tom Thumb
- Dandelion (*Taraxacum officinale* Weber)
- Annual marguerite (*Chrysanthemum coronarium* L.)
- Shasta daisy (*Chrysanthemum maximum* Ram.)
- English daisy (*Bellis perennis* L.)
- China-aster (*Callistephus chinensis* Nees) var. Giant Branching White, wilt resistant.
- French marigold (*Tagetes patula* L.)
- African marigold (*Tagetes erecta* L.)
- Winter cape-marigold (*Dimorpholheca aurantiaca* DC.)
- *Gaillardia pulchella* Foug. var. *picta* Gray
- Hybrid cineraria (*Senecio cruentus* DC.)

Cruciferae:
- Rape (*Brassica napus* L.)
- Rutabaga (*Brassica campestris* L. var. *napobrassica* DC.)
- Annual stock (*Matthiola incana* R. Br. var. *annua* Voss) var. Fiery Blood Red
- Honesty (*Lunaria annua* L.)
- Dames violet (*Hesperis matronalis* L.)
- *Brassica integrifolia* O. E. Schulz var. *chevalieri* R. Porteres

Cucurbitaceae:
- Cucumber (*Cucumis sativus* L.)

Dipsacaceae:
Mourning bride or pincushion flower (*Scabiosa atropurpurea* L.)
Euphorbiaceae:
Castor-bean (*Ricinus communis* L.)
Geraniaceae:
Storksbill (*Pelargonium zonale* Willd.)
Gramineae:
Corn (*Zea mays* L.) var. Golden Bantam
Labiatae:
Flowering sage (*Salvia farinacea* Benth.)
Leguminosae:
Garden pea (*Pisum sativum* L.) var. Alderman
Broadbean (*Vicia faba* L.)
Lobeliaceae:
Lobelia (*Lobelia hybrida* Hort.)
Onagraceae:
Clarkia elegans Dougl.
Godetia grandiflora Lindl.
Papaveraceae:
Iceland poppy (*Papaver nudicaule* L.)
Oriental poppy (*Papaver orientale* L.)
Polygonaceae:
Rhubarb (*Rheum rhaponticum* L.)
Resedaceae:
Mignonette (*Reseda odorata* L.)
Rosaceae:
Geum chiloense Balb.
Scrophulariaceae:
Snapdragon (*Antirrhinum majus* L.)
Pentstemon or beardtongue (*Pentstemon barbatus* Nutt.)
Solanaceae:
Solanum aviculare Forst.
Potato (*Solanum tuberosum* L.)
Tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey) var. Early Santa Clara Canner
Current tomato (*Lycopersicum pimpinellifolium* Dunal)
Jimsonweed (*Datura stramonium* L.)
Petunia (*Petunia hybrida* Hort.)
Tropaeolaceae:
Garden nasturtium (*Tropaeolum majus* L.)
Umbelliferae:
Celery (*Apium graveolens* L.)
Verbenaceae:
Garden verbena (*Verbena hybrida* Voss)
Violaceæ:
Pansy (*Viola tricolor* L.)

Subsequent inoculations to radish with extracted juice from inoculated plants of the above-mentioned plant species failed to cause infection.

PROPERTIES OF THE VIRUS

The results of property studies of the radish mosaic virus are shown in table 2. Virus samples, consisting of 2 cc of undiluted juice in small, thin-walled test tubes, were used in determining resistance to aging in vitro and inactivation temperature. In determining the tolerance to dilution, the virus was diluted with the required amount of distilled water. Young White Icicle radish plants were then inoculated mechanically with the different virus samples.

Samples of the radish mosaic virus were infectious for 14 days after aging in vitro at 22° C., but were inactivated after 16 days. The virus produced infection after heating for 10 minutes at 65°, but was inactivated at 68°. A dilution tolerance of 1 to 14,000 was established. In each of the three tests, 25 noninoculated radish plants served as controls and they remained healthy.

TABLE 2.—*Longevity in vitro, inactivation temperature, and tolerance to dilution of the radish mosaic virus*

[5 trials made with 25 plants each in all instances]

LONGEVITY IN VITRO, 22° C.

Aged (hours)	Plants infected	Aged (hours)	Plants infected	Aged (hours)	Plants infected	Aged (hours)	Plants infected
	Number		*Number*		*Number*		*Number*
0	25	72	24	144	22	336	5
24	25	96	24	168	23	384	0
48	25	120	25	240	18	432	0

INACTIVATION TEMPERATURE (10 MINUTES)

Temperature (°C.)	Plants infected	Temperature (°C.)	Plants infected	Temperature (°C.)	Plants infected	Temperature (°C.)	Plants infected
	Number		*Number*		*Number*		*Number*
50	25	60	20	68	0	75	0
55	25	65	7	70	0	Untreated	25

TOLERANCE TO DILUTION

Dilution	Plants infected	Dilution	Plants infected	Dilution	Plants infected	Dilution	Plants infected
	Number		*Number*		*Number*		*Number*
0	25	1:2,000	23	1:7,000	10	1:12,000	3
1:10	24	1:3,000	19	1:8,000	9	1:13,000	3
1:100	25	1:4,000	16	1:9,000	7	1:14,000	1
1:500	22	1:5,000	13	1:10,000	7	1:15,000	0
1:1,000	24	1:6,000	14	1:11,000	3	1:16,000	0

DESCRIPTION OF THE RADISH MOSAIC VIRUS

Transmissible by mechanical inoculation with or without powdered carborundum. Incubation period 9 to 18 days. Resistance to aging in vitro between 14 and 16 days. Inactivation temperature between 65° and 68° C. for a 10-minute exposure. Tolerance to dilution approximately 1 to 14,000. White radish (*Raphanus sativus*) and certain other vegetable and ornamental crucifers susceptible. Small, chlorotic lesions produced on White Icicle radish, followed by medium type of mottling. Mottled leaves later show marked bleaching. Occasional distortion and rugosity of leaves in late stages of infection. Virus lethal to Virginian stock and evening scented stock; occasionally infected spinach plants die. Local chlorotic lesions produced on leaves of *Nicotiana glutinosa* and *N. tabacum;* systemic chlorotic rings on *N. rustica* var. *humulis*, and systemic chlorotic and necrotic lesions on *N. langsdorffii.*

COMPARISON OF THE RADISH MOSAIC VIRUS WITH CERTAIN OTHER CRUCIFER VIRUSES

The radish mosaic virus can readily be differentiated from the mosaic viruses of cauliflower (*12*), Chinese cabbage (*15*), turnip (*13*), and annual stock (*11*), and from the black ring virus of cabbage (*14*) when compared as to symptomatology, experimental host range, and properties. In making these comparisons, both on radish and other plants, parallel series of mechanical inoculations were made simultaneously in the greenhouse. Because of space limitations, only a few of the most conspicuous differences can be discussed in detail.

On young White Icicle radish plants, the symptoms produced by the radish mosaic virus cannot be confused with those produced by the other crucifer viruses mentioned above. The cauliflower mosaic virus

causes pronounced vein clearing on the leaves of this host; the Chinese cabbage mosaic virus, vein clearing and a fine type of mottling; the mild annual stock mosaic virus, small, chlorotic lesions; while the severe annual stock mosaic virus, the turnip mosaic virus, and the black ring virus of cabbage failed to cause infection.

Marked differences in symptomatology were observed on certain other cruciferous plants in these comparative tests. For example, the symptoms of the radish mosaic virus on cabbage and cauliflower consist of numerous, somewhat diffuse, large, chlorotic lesions (fig. 4, *A*, *B*). In contrast, the cauliflower mosaic virus induced vein clearing on cabbage and vein clearing, vein banding, mottling, and necrotic lesions on cauliflower; the Chinese cabbage mosaic virus, a coarse, yellowish vein banding on cabbage and chlorotic lesions on cauliflower which are usually only visible in transmitted light; the turnip mosaic virus, chlorotic lesions with necrotic edges on cabbage but only large, diffuse, chlorotic lesions on cauliflower; and the black ring virus of cabbage, both chlorotic and necrotic lesions and marked general chlorosis on leaves of cabbage, and only chlorotic lesions on cauliflower. No infection was obtained on cabbage and cauliflower with the mild and severe mosaic viruses of annual stock.

On Chinese cabbage, the radish mosaic virus induced chlorotic and necrotic lesions with vein necrosis on inoculated leaves only. The cauliflower mosaic virus caused vein clearing; the Chinese cabbage mosaic virus, vein clearing and mottling; the turnip mosaic virus, coarse yellow vein banding and leaf distortion; the mild mosaic virus of annual stock, a mild, diffuse, and somewhat coarse type of mottling; and the black ring virus of cabbage, chlorotic lesions, some of which later become necrotic. The severe mosaic virus of annual stock did not cause infection of Chinese cabbage.

Purple Top White Globe turnip plants were only occasionally infected by the radish mosaic virus, the symptoms consisting of systemic mottling and severe leaf distortion. In comparison, the cauliflower mosaic virus caused vein clearing on the leaves of this host; the Chinese cabbage mosaic virus caused vein clearing and a fine type of mottling; the turnip mosaic virus, vein clearing and mottling with numerous, raised, dark-green islands; the mild mosaic virus of annual stock, a fine type of mottling; and the black ring virus of cabbage, chlorotic lesions, some of which became necrotic. Turnip plants were not infected by the severe mosaic virus of annual stock.

Other important differences between the radish mosaic virus and the other crucifer viruses mentioned are as follows: No infection of annual stock plants was obtained in numerous tests with the radish mosaic virus. The cauliflower mosaic virus induced marked vein clearing but no flower breaking on annual stock, whereas the Chinese cabbage, turnip, and cabbage black ring viruses caused leaf mottling and flower breaking. The radish mosaic virus is lethal to evening scented stock, Virginian stock, and occasionally to spinach. Large, diffuse, chlorotic lesions were produced only on inoculated leaves of Turkish tobacco and *Nicotiana glutinosa*, and systemic infection of rocket larkspur was obtained. Other crucifer viruses did not produce similar results.

Further, the radish mosaic virus may be differentiated from the other crucifer viruses included in these studies by its inactivation

temperature (65° to 68° C.) and its greater tolerance to dilution (1 to 14,000).

SUMMARY

A mosaic disease of radish in the San Francisco Bay section of California is described.

The symptoms of the disease consist at first of irregular-shaped, chlorotic lesions which later develop into a coarse mottle. On older, infected plants, the normal, dark-green tissue appears as irregularly shaped, nonraised islands on a yellowish-green, chlorotic background.

The radish mosaic virus is readily transmissible by mechanical inoculation, with or without carborundum. The incubation period ranges from 9 to 18 days. Unsuccessful attempts were made in the greenhouse to transmit the radish mosaic virus by means of the cabbage, green peach, or turnip aphids.

The virus retained its infectivity after aging for 14 days at 22° C. It is inactivated by heating for 10 minutes at 68°, but causes infection when diluted up to 1, to 14,000.

The host range of the radish mosaic virus includes 19 species of plants representing 9 genera in 4 families. In the family Cruciferae, infection was obtained on pe-tsai, kale, Brussels sprouts, cabbage, sprouting broccoli, cauliflower, kohlrabi, black and white mustard, evening scented stock, Virginian stock, turnip, Chinese mustard, and Chinese radish, in addition to several cruciferous weeds. Annual stock plants were found to be resistant. Other susceptible hosts included lambsquarters, sowbane, spinach, rocket larkspur, *Nicotiana glutinosa*, *N. langsdorffii*, *N. rustica* var. *humulis*, and Turkish and White Burley tobacco (*N. tabacum*).

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SEASONAL VARIATION IN TANNIN CONTENT OF LESPEDEZA SERICEA[1]

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INTRODUCTION

Leguminous plants inexpensive to grow and capable of yielding profitable forage and hay crops are particularly needed in the warm humid sections of the southeastern part of the United States, where the amount of organic matter in the soil is small. During the last few years observations at the various agricultural experiment stations in this area have indicated that *Lespedeza sericea* Benth., a foreign plant introduction, is unusually well adapted to many localities where such conditions prevail (*9; 13; 15, pp. 65–85*).[2] This perennial legume is particularly resistant to drought and disease and produces high yields of green forage or easily cured hay. It can be grown on certain poor, acid soils without benefit of lime or fertilizer.

The results of feeding livestock with *Lespedeza sericea*, either as hay or green forage, have been conflicting. In some cases, such feeding has adversely affected the health and milk production of cattle, and at times the cattle have refused to eat the plant.

Mooers and Ogden (*13, pp. 18–19*) report the experience of several farmers who found that sericea hay was eaten as readily as Korean lespedeza and that it could be fed with satisfactory results. Hartman (*8, p. 127*) states that *Lespedeza sericea* apparently was grazed by cattle as much as other vegetation in the same pasture. Grinnels (*7*), on the other hand, in a feeding experiment in which *L. sericea* was compared with alfalfa, observed that the former seemed less palatable, that the animals fed the sericea ration lost considerable weight, and that those fed the alfalfa ration produced 18.3 percent more milk and butter.

In feeding trials with *Lespedeza sericea* started in 1935 at the Kentucky Agricultural Experiment Station (*11*), 10 ewes lost 99 pounds in 28 days, and it became impossible to continue feeding the hay. In 1936, however, 10 ewes lost only 32 pounds in 28 days, from which it was concluded that there probably was a difference in the quality of the hay fed in the 2 years.

Holdaway et al. found that the milk production of cows fed *Lespedeza sericea* was about 80 percent of that of cows fed alfalfa, and they state (*10, p. 4*): "The Lespedeza sericea hay was less readily eaten than either Korean lespedeza or alfalfa. * * * While cows may not like the lespedeza hays for the first few days of feeding, they soon become accustomed to them."

[1] Received for publication June 14, 1938. Cooperative investigation of the Industrial Farm Products Research Division, Bureau of Chemistry and Soils, and the Division of Forage Crops and Diseases, Bureau of Plant Industry.

[2] Italic numbers in parentheses refer to Literature Cited, p. 138.

Kinney, Kenney, and Fergus (*12, p. 7*) say of *Lespedeza sericea*: "It produces good yields of hay but feeding tests have given conflicting results as regards its feeding value. Certainly it must be cut at an early stage of growth to be palatable."

As to the present status of *Lespedeza sericea* Pieters [3] says:

> A tentative conclusion from all information available today is that *L. sericea* cut at the right time and well cured makes good hay and a satisfactory roughage for beef cattle and work stock, but that some grass hay or straw should be fed with it. This is especially true for dairy cattle.

It is evident that sometimes something is present in *Lespedeza sericea* in sufficient quantity to lower its palatability and interfere with its digestion by livestock. One constituent that might be responsible for these objectionable features is tannin, since it is distasteful and astringent. An appreciable variation in the tannin content might explain the conflicting results with the feeding of this plant.

Helm and Etheridge (*9, p. 13*) state: "The growing crop contains from 5 to 9 per cent tannin, which may partly explain why the animals do not at first graze the plant readily." Blair (*4, p. 3*) states that "the leaves are also said to be injurious to some classes of livestock on account of their high tannic acid content."

Ogden (*14, p. 12*) says: "There is an astringent quality thought to be associated with the high content of tannin in sericea." A colorimetric test showed that *Lespedeza sericea* plants contained from 2.2 to 10 per cent of tannin.

During the past 6 years numerous unpublished analyses by the Bureau of Chemistry and Soils have shown that *Lespedeza sericea* contains appreciable quantities of tannin. From these analyses Pieters (*15, p. 82*) gives 5.92 per cent as the lowest tannin content of the whole plant, and 9.77 per cent as the highest.

No data on the tannin content of *Lespedeza sericea* other than that already cited have been found. The studies described here present, so far as the authors are aware, the first quantitative data on the seasonal variation in the tannin content of *L. sericea*. The period covered is that part of the season in which *L. sericea* would be suitable for hay.

PLANT MATERIAL

Plant material for this study was obtained in 1935 from a 4-year-old stand of *Lespedeza sericea* No. 04730 at the Arlington Experiment Farm of the United States Department of Agriculture, at Arlington, Va. It was grown from seed drilled on winter wheat in the spring of 1931. The field was a poorly drained and rather impervious clay loam. It was low in fertility and had received no fertilizers. Seed crops were gathered from the stand in 1932 and 1933 and a hay crop in 1934.

The growth in 1935 was produced under normal conditions of temperature and rainfall. Actual forage yields were not recorded since the material was not removed from an area of definite size. However, yields of sericea hay taken under comparable conditions average about 2,500 pounds per acre of moisture-free material on June 1, and about 5,000 pounds per acre on August 1.

[3] PIETERS, ADRAIN J. LESPEDEZA SERICEA. U. S. Bur. Plant Indus. 12 pp. 1937. [Mimeographed.]
See p. 5.

The average height of the plants on May 29, when sampling was started, was about 19 inches. For the 3 succeeding weeks the average height was 22, 28, and 34 inches, respectively. After June 20, gain in height slowed down appreciably, the cutting made on July 24 averaging 40 inches. Because of shade from the leafy top growth, some lower leaves dropped before the late July cuttings were made.

Approximately 8 pounds of green material, cut by hand, was gathered at weekly intervals from May 29 to July 31 inclusive. A stubble was left comparable in height to that from ordinary mowing. All samples were first cuttings. No samples were taken after the appearance of the first blooms.

All cut material was placed immediately in shrinkage bags and weighed. The bags were then hung in a warm shaded room and the contents allowed to air-dry for 3 to 4 weeks before leaves and stems were separated. The percentage of dry matter in the fresh plant and the proportion of leaves and of stems, in the moisture-free plant are given for each cutting in table 1. Moisture was determined by oven-drying at 100° to 103° C.

TABLE 1.—*Percentage of dry matter, leaves, and stems in Lespedeza sericea cut at weekly intervals in 1935 at the Arlington Experiment Farm, Arlington, Va.*

Date harvested	Dry matter in fresh plant	Leaves in moisture-free plant [1]	Stems in moisture-free plant [1]	Date harvested	Dry matter in fresh plant	Leaves in moisture-free plant [1]	Stems in moisture-free plant [1]
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
May 29		61.6	38.4	July 3	47.7	48.2	51.8
June 5	33.5	65.5	34.5	July 10	49.7	43.8	56.2
June 12	30.0	50.9	49.1	July 17	50.9	44.6	55.4
June 20	40.2	55.8	44.2	July 24	51.4	44.5	55.5
June 26	42.3	49.6	50.4	July 31	56.4	42.8	57.2

[1] Separation of leaves and stems was made by the Bureau of Agricultural Economics, United States Department of Agriculture.

Leafiness (table 1) decreased with the age of the plant, the percentage of leaves in the last cutting being only about two-thirds that in the first. The percentage of total dry matter increased as the plant matured, and the rate of increase was about the same as that for stems.

QUALITATIVE EXAMINATION FOR TANNIN

Numerous qualitative tests for tannin in plant materials have been proposed, but none appears to be infallible. Positive results are sometimes obtained with constituents that are not tannin. However, failure of the various tests at times is not surprising since all the tannins are not chemically identical. Water extracts of *Lespedeza sericea* were examined by several tests. In every case a positive response for tannin was obtained. The goldbeater's-skin test (*16*), which is one of the most dependable, gives a decided positive reaction. Sericea extracts form heavy precipitates with the gelatin-salt reagent (*3, p. 136*) and with solutions of quinine, cinchonine, and antipyrine. Iron ammonium citrate, recommended by Ware (*17*) as an apparently specific test for tannin, gives a typical heavy black precipitate.

The natural tannins have been divided into two classes, the pyrogallol and the catechol tannins (*3, pp. 135–141*). *Lespedeza sericea* tannin belongs to the catechol class, as is shown by the fact that it

gives an olive-green color with iron-alum solution and a precipitate with bromine water, and is completely precipitated by formaldehyde in the presence of hydrochloric acid.

METHODS OF TANNIN ANALYSES

The tannin content was determined by the hide-powder method of the American Leather Chemists' Association (*1, pp. 46, 48–50; 2, pp. 119–120*) modified slightly as to amount of sample extracted and hide powder used for removal of tannin. By this method the solution containing the tannin is shaken with lightly chromed hide powder and filtered, and an aliquot is evaporated. Tannin is taken as the difference between the dry matter in this residue and that from a corresponding amount of the clear solution not shaken with hide powder. This is designated total tannin.

Tannin was also determined by the revised Wilson-Kern method (*19, pp. 290–295*), in which the tanned hide powder is washed thoroughly with water, then dried and weighed. Tannin is calculated from the increase in weight of the hide powder. This tannin is quite firmly combined with the hide powder, and as it is not readily washed out it is sometimes referred to as irreversibly fixed. It will be spoken of herein as fixable tannin to distinguish it from total tannin. The Wilson-Kern method gives markedly lower values than the hide-powder method of the American Leather Chemists' Association. In some cases figures for tannin by the Wilson-Kern method may be only one-half or even one-third as much as by the latter method.

The difference in tannin content shown by the two methods is related to astringency. In general, the most astringent materials show the least difference. In fact, the ratio of tannin by the Wilson-Kern method to that by the American Leather Chemists' Association method, or the ratio of fixable to total tannin, has been proposed as a measure of astringency (*5*). Astringency is herein considered as approximately defined by this ratio. The tannin is referred to as astringent, although this property seems to depend upon the kind and amount of associated nontannins as well as upon the nature of the tannin.

In preparing the extract solutions for analysis, a quantity of leaves was taken that would give 4 ± 0.25 g of total tannin per liter. A quantity of the stems sufficient to give a tannin concentration of 4 g per liter could not be used, so 50-g portions were extracted. All extractions were made in glass extractors (*6*). The first 500 cc of the percolate was collected in 2 hours without refluxing, and then the extraction was continued under a reflux condenser for 14 hours with 500 cc of water. The two portions of the extract were combined, heated to 80° C., cooled rapidly to 20°, made to volume, and analyzed at a room temperature of 20°.

Total solids, soluble solids, and nontannins in the extract were determined by the method of the American Leather Chemists' Association (*1, 2*). In determining nontannins, 46 g of wet, chromed hide powder per 200 cc of extract solution was used for the leaves and 5 or 10 g for the stems.

In determining fixable tannin by the Wilson-Kern method (*19*), the special, washed hide powder was tanned with the extract solution for 24 hours, being shaken for 1 or 2 hours at the beginning and 5 hours at the end of this period. The tanned powder was then washed

overnight with 10 or 12 liters of water flowing through at the rate of 2 or 3 drops per second. After it was dried, the gain in weight was calculated as tannin.

The values for the formaldehyde-hydrochloric acid precipitate were obtained by boiling 50 cc of the extract solution with 5 cc of hydrochloric acid and 10 cc of 40-percent formaldehyde for 30 minutes under a reflux condenser. The cooled solution was filtered through a Jena fritted-glass crucible No. 2 G 3/7. The precipitate was washed well with hot water, dried at 100° C., and weighed.

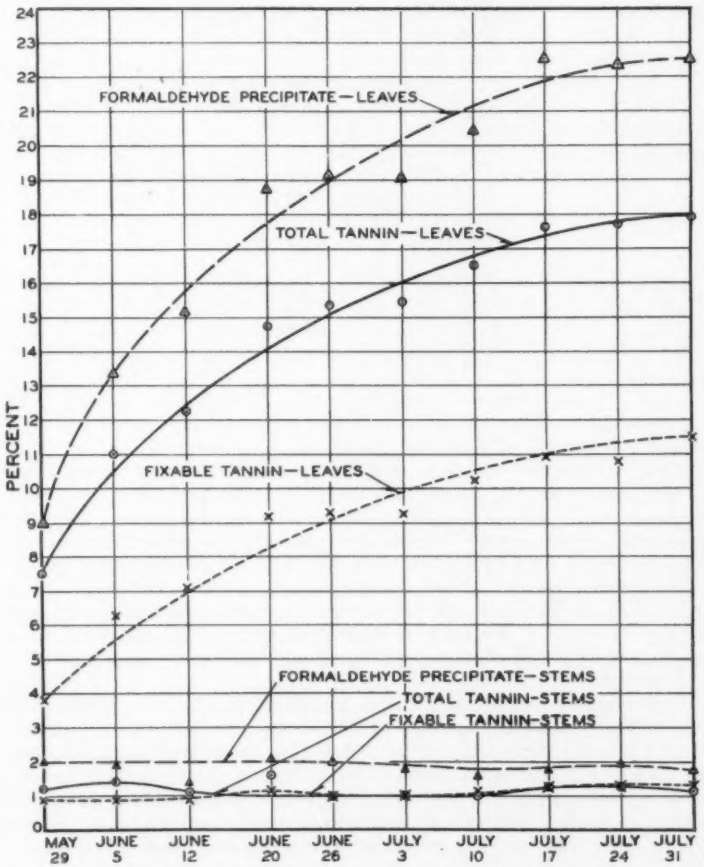

FIGURE 1.—Seasonal variation in the tannin content of *Lespedeza sericea*.

ANALYTICAL RESULTS

The analytical results are given in table 2 and figure 1. The values for the whole plant were calculated from the figures for the leaf and stem portions.

TABLE 2.—*Tannin content of Lespedeza sericea hays*

[On moisture-free basis]

Portion of plant	Date harvested in 1935	Total solids in extract	Soluble solids in extract	Non-tannins	Total tannin	Fixable tannin	Formaldehyde precipitate
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Leaves	May 29	25.0	23.2	15.7	7.5	3.8	9.0
	June 5	30.0	27.7	16.7	11.0	6.3	13.4
	June 12	31.4	28.9	16.6	12.3	7.1	15.2
	June 20	36.8	33.3	18.5	14.8	9.2	18.8
	June 26	37.5	34.0	18.6	15.4	9.3	19.2
	July 3	37.8	34.8	19.3	15.5	9.3	19.1
	July 10	39.5	36.5	19.9	16.6	10.3	20.5
	July 17	41.4	38.4	20.7	17.7	11.0	22.6
	July 24	40.8	38.2	20.4	17.8	10.9	22.4
	July 31	43.2	40.1	22.1	18.0	11.6	22.6
Stems	May 29	24.7	23.6	22.4	1.2	.9	2.0
	June 5	18.6	17.4	16.0	1.4	.9	1.9
	June 12	17.9	16.7	15.6	1.1	.9	1.4
	June 20	16.5	15.7	14.1	1.6	1.2	2.1
	June 26	16.6	15.7	14.7	1.0	1.0	2.0
	July 3	16.2	15.4	14.4	1.0	1.0	1.8
	July 10	15.3	14.6	13.6	1.0	1.1	1.6
	July 17	15.8	14.9	13.6	1.3	1.3	1.8
	July 24	14.6	14.0	12.6	1.3	1.3	2.0
	July 31	15.4	14.3	13.1	1.2	1.3	1.8
Whole plant	May 29	24.9	23.4	18.3	5.1	2.7	6.3
	June 5	26.1	24.1	16.5	7.7	4.4	9.4
	June 12	24.8	22.9	16.1	6.8	4.1	8.4
	June 20	27.8	25.5	16.6	9.0	5.7	11.4
	June 26	27.0	24.8	16.6	8.1	5.1	10.5
	July 3	26.6	24.8	16.8	8.0	5.0	10.1
	July 10	25.9	24.2	16.4	7.8	5.1	9.9
	July 17	27.2	25.4	16.8	8.6	5.6	11.1
	July 24	26.3	24.8	16.1	8.6	5.6	11.1
	July 31	27.1	25.3	17.3	8.0	5.7	10.7

In the leaves the content of soluble solids increased steadily. At the end of July it was nearly double that at the end of May. The quantity of soluble solids in the stems decreased, but after June 5 the decrease was not great. Consequently, in the plant as a whole there were only minor irregular changes in soluble solids. The proportion of soluble solids derived from the leaves and from the stems was also nearly constant, except for the first one or two samples.

The tannin content of the leaves increased markedly with the age of the plant. The total tannin content rose rapidly from 7.5 percent on May 29 to 15.4 percent, or more than twice as much, on June 26, and then slowly increased until it reached the surprisingly high figure of 18 percent.

Fixable tannin in the leaves also increased during the growth of the plant. The gain was rapid at first, rising from 3.8 percent on May 29 to 9.2 percent on June 20. After this date the rate of increase was slight, but on July 31 the fixable tannin reached 11.6 percent, which is rather a high value for this type of tannin. Many recognized tanning materials contain no more fixable tannin than this. Wilson (*18, p. 434*), for example, reports figures which, when calculated to the moisture-free basis, show only 6.77 percent of fixable tannin in hemlock bark and 10.59 percent in sumac leaves.

In the last sample, cut July 31, the leaves contained about two and one-third times as much total tannin and three times as much fixable tannin as the first cutting on May 29. In the earliest sample about half the total tannin was fixable, while in the last one about two-thirds was fixable. Thus, not only the tannin content of the leaves,

but also the astringency of the tannin increases as the season advances.

The total tannin content of the stems was quite low, just over 1 percent, and it changed very little with age. Fixable tannin was practically equal to total tannin, so that although the stems contained very little tannin it was markedly astringent.

Because of the decrease in leafiness of the plant with increase in the tannin content of the leaves a balance was apparently reached, whereby after the first of June the percentage of total tannin in the whole plant was essentially constant. Fixable tannin in the whole plant was low at first, but on June 20 it was about twice as much as on May 29. After June 20 there was little change. The values for the formaldehyde precipitate and total tannin closely parallel each other. The ratio of formalydehde precipitate to total tannin ranged from 1.2 to 1.3 for the leaves and whole plant. It averaged 1.25 for the former and 1.27 for the latter. For stems the ratio is somewhat higher, averaging about 1.55. Under certain circumstances, therefore, the determination of the formaldehyde precipitate might be used as a convenient procedure for determining the approximate tannin content of the leaves or whole plants.

SUMMARY AND CONCLUSIONS

From a plot of *Lespedeza sericea* (No. 04730) growing at the Arlington Experiment Farm, at Arlington, Va., first cuttings were taken for examination at weekly intervals from May 29 to July 31.

The percentage of leaves in the whole plant decreased from 61.6 on May 29 to 42.8 on July 31. Soluble matter in the leaves nearly doubled during this period. That in the stems decreased. In the plant as a whole there was little change in content of soluble matter.

Total tannin in the leaves increased from 7.5 to 18 percent, and fixable tannin increased from 3.8 to 11.6 percent. The last cutting, therefore, contained about two and one-third times as much total tannin and three times as much fixable tannin as the first cutting. Not only the quantity of tannin in the leaves but also the astringency of the tannin increased during spring and summer. Therefore, if tannin is an important factor in palatability cattle should find the leaves of *Lespedeza sericea* progressively less attractive during these periods. Adverse effects upon the health of cattle caused by digestive disturbances from the intake of excessive quantities of tannin should also occur more generally during this time.

The quantity of total tannin in the stems was relatively insignificant, ranging only from 1 to 1.6 percent. In the whole plant the percentages of total and fixable tannin were essentially constant except for the three earliest cuttings. Palatability, therefore, might be expected to be the same during the last of June and July. However, in a heterogeneous mixture of this kind it may be that dilution of the leaves with the drier and more fibrous material does not cause a proportional decrease in astringency.

The formaldehyde precipitation method gives results differing from those for total tannin by a constant factor. Under certain circumstances, therefore, this method might be used as a convenient procedure for determining the approximate tannin content of *Lespedeza sericea*.

The data presented on tannin content offer an entirely plausible explanation of apparently contradictory experiences with the feeding of *Lespedeza sericea*. Tannin may or may not be the dominant factor, depending on the time of year and the stage of growth of the plant. However, to study other factors and to prove the relationship between tannin content, palatability, and digestibility numerous careful observations on the results of feeding *L. sericea* of different tannin content would be necessary.

There is an increasing appreciation of the role that tannin might play in jeopardizing the future development of *Lespedeza sericea* as an important forage and hay crop. A collaborative program of research on tannin in this plant by the Bureaus of Plant Industry and Chemistry and Soils has as two of its major objectives the determination of the influence of environmental factors on the tannin content and the selection and breeding, if possible, of strains of *L. sericea* containing little or no tannin.

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PSEUDOMONAS AERUGINOSA IN BOVINE MASTITIS [1]

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INTRODUCTION

Small, Gram-negative bacteria that produced a blue-green fluorescence characteristic of *Pseudomonas aeruginosa* (*Bacillus pyocyaneus*) were found in all but 2 cases of an unusually severe outbreak of bovine mastitis involving 23 cows. Since the outbreak was unusual in character, a study was carried on to determine the identity of the organisms and their possible significance. The results are reported in this paper.

REVIEW OF LITERATURE

The occurrence of *Pseudomonas aeruginosa* in the mammary glands has seldom been reported. In fact, Carpenter (*3*) [3] states that he attempted artificial inoculation of a normal udder with *Bacterium pyocyaneum* without causing more than temporary mild symptoms of mastitis. Natural invasion of the udder was indicated when Mundhenk (*13*) found *Bacillus pyocyaneus* in milk samples from cows with severe cases of mastitis, but he considered the organisms only secondary invaders associated with colon bacilli.

Pickens, Welch, and Poelma (*15*) studied an outbreak of mastitis that they attributed to *Pseudomonas aeruginosa*, in which agglutination tests with *Ps. aeruginosa* antigen on blood from cows with infected udders showed titers as high as 1:1,000 in some cases, whereas the blood from cows with normal udders showed titers of 1:50 or less. Some of the cows had periodic relapses, but gave apparently normal milk between attacks. Cherrington and Gildow (*4*) also reported recurring attacks of acute mastitis in which *Ps. aeruginosa* was found in the milk in almost pure culture. Their findings were likewise supported with agglutination tests. They attributed the recurrent attacks to a water supply contaminated with *Ps. aeruginosa*.

NATURE OF THE OUTBREAK

The outbreak reported herein occurred in the Bureau of Dairy Industry herd at Beltsville, Md., where samples of milk were being collected periodically for examination for indications of mastitis. Small, Gram-negative rods giving the characteristic green fluorescence of *Pseudomonas aeruginosa* were first isolated on March 29, 1937 from milk from the left front quarter of cow 1247. An abrupt rise in

[1] Received for publication September 14, 1938.

[2] The author acknowledges the cooperation of W. T. Miller, associate veterinarian, Bureau of Animal Industry, in supplying some of the strains used in this study and the data on the temperatures of the affected cows; of M. H. Fohrman and W. J. Sweetman, both of the Bureau of Dairy Industry, in supplying the production records and assisting in the collection of the samples.

[3] Italic numbers in parentheses refer to Literature Cited, p. 00.

chloride content and leucocyte count of the milk from the infected quarter indicated a mild case of mastitis.

On April 24 cow 880 suffered a sudden attack of mastitis. Her milk production dropped from a normal of 65 pounds on the previous day to 30 pounds, but gradually returned to almost normal in 4 days. A milk sample from the left rear quarter taken April 27 yielded many green fluorescent, Gram-negative rods. On May 3 the organisms were isolated from a third cow that showed mild symptoms of mastitis.

On May 10 the disease broke out in epizootic proportions. Between then and June 11 milk samples from the infected quarters of 17 cows suffering from severe mastitis were found to contain the green fluorescent rods in apparently pure culture. Many of the affected animals were obviously sick, one animal running a temperature as high as 106.8° F. Milk production dropped abruptly, in some cases to almost nothing. The milk from the affected quarters was watery, yellow, and very lumpy and stringy. The number of pus cells, as obtained in a direct smear, were estimated to be more than 50,000,000 per milliliter. The records of cows A–116 and A–118 (table 1) well illustrate the course of the disease.

TABLE 1.—*Milk-production records of cows A–116 and A–118*

Date	Milk production of Cow A–116 [1]			
	Morning milking	Noon milking	Evening milking	Total
	Pounds	*Pounds*	*Pounds*	*Pounds*
May 14				30.1
May 15				30.2
May 16	11.0	11.9	8.3	31.2
May 17	11.5	7.0	5.4	23.9
May 18	4.7	4.4	2.7	11.8
May 19	6.9	4.6	1.3	12.8
May 20	5.8	6.7	4.1	16.6
May 21	7.4	7.8	5.0	20.2
May 22	8.9	8.5	6.3	23.7
May 23	10.0	8.0	7.2	25.2
May 30	10.6	10.4	7.7	28.7

Date	Milk production of cow A–118 [2]			
	Morning milking	Noon milking	Evening milking	Total
	Pounds	*Pounds*	*Pounds*	*Pounds*
May 29	14.2	14.3	13.4	41.9
May 30	15.6	16.3	11.1	43.0
May 31	11.2	16.0	3.7	30.9
June 1	4.5	3.6	0.4	8.5
June 2	2.0	0.2	0.4	2.6
June 3	1.5	1.6	0.5	3.6
June 4	2.8	3.7	2.4	8.9
June 5	5.8	5.3	4.0	15.1
June 8	14.0	6.4	4.7	25.1
June 15	12.1	9.2	6.0	27.3

[1] Rectal temperature: May 18, 106° F.; May 24, 103°; May 25, 102.4°. Bacteriological examination: Green fluorescent rods were found in the milk for May 18, 22, and June 4; none was found in milk of June 9.

[2] Rectal temperature: May 31, 106.7° F.; June 4, 103°. Bacteriological examination: Green fluorescent rods were found in the milk May 31, June 1, 4, 9, 11, and 18; none was found in milk of Aug. 16 and Dec. 13. A year after the onset of the disease the quarter was still hard and swollen, and the milk was alkaline to bromocresol purple.

As a result of the infection one cow lost a quarter permanently and another cow lost one temporarily. With some of the other cows the affected quarters have remained more or less hard and swollen, although producing milk of apparently normal appearance. For most of the cows few data are available regarding the persistence with which the green fluorescent rods were shed into the milk after the acute attack had cleared up. Only two of the infected cows (1441 and 1445) remained on regular semimonthly sampling. When cow 1441 freshened on December 13, all four quarters were producing. At that time and again on February 28, 1938, she had recurrences of mild mastitis, with large numbers of the organisms present in the milk. Cow 1445 continued shedding the organisms into the milk until she went dry in October. After freshening again on January 9, 1938, she continued to suffer mild recurrences of mastitis in the affected quarter and the organisms were found in the milk on January 24, February 28, and May 23. The milk from that quarter has continued to be higher in chloride content and leucocyte count and slightly more alkaline to bromocresol purple than the milk from her normal quarters.

Only one new case of mastitis was found after June 11, 1937. A sample of milk taken August 31 from the right rear quarter of cow 1236 showed a few of the green fluorescent rods and a slight elevation of the chloride content and the leucocyte count. She showed no further disturbances from then until she was removed from the herd in December.

CHARACTERISTICS OF THE ORGANISMS ISOLATED

A pure-culture study of 18 strains of the green fluorescent organisms isolated from the infected quarters showed that their characteristics agree quite closely with those described in Bergey's Manual (*1*) for *Pseudomonas aeruginosa* except in two important features—their failure to produce indole and their ability to produce acid from certain carbohydrates. Bergey considered indole production sufficiently constant to justify the use of this characteristic in his key to separate *Ps. aeruginosa* from those species not producing indole.

A search of the literature on *Pseudomonas aeruginosa*, however, revealed an extreme lack of agreement on both indole production and sugar fermentations. Chester (*5*) described the species as indole-negative. Pickens and coworkers (*15*), Cherrington and Gildow (*4*), Buonomini (*2*), Fuller and Johnson (*7*), and Stark and Scheib (*20*) found no indole production. Jordon (*10*) found three out of seven strains producing indole and four not producing it. Lartigau (*11*) and Růžička (*16*) found that indole was produced. Sherwood, Johnson, and Radotincky (*18*) studied 22 strains obtained from various laboratories and found that all produced indole with Nelson's (*14*) vanillin test.

In addition to the 18 strains isolated from milk produced by cows affected with mastitis, 4 strains of *Pseudomonas aeruginosa* obtained from other laboratories (including No. 256 and No. 914 from the American type culture collection) were tested for indole production. The organisms were grown for 72 hours at 37° C. in Bacto-tryptophane broth. Determinations for indole were made by the Goré test and the Ehrlich-Böhme test described in the Manual of Methods for Pure Culture Study (*19*) and by the vanillin test of Nelson (*14*). None of

the strains tested gave a positive reaction by any of the three methods.

The reason for the conflicting reports regarding the ability of *Pseudomonas aeruginosa* to ferment carbohydrates is more understandable than in the case of indole production. Sears and Gourley (*17*) have shown that certain carbohydrates are utilized by *Ps. aeruginosa*, but that the production of an acid reaction depends upon the nitrogen content of the medium. They found that in a glucose broth containing 1 percent or less of peptone the glucose was attacked and the reaction dropped to between pH 5.0 and 4.0. When higher concentrations of peptone were employed, the production of ammonia from the break-down of the peptone was sufficiently rapid to neutralize the acid produced from the glucose and keep the reaction of the medium neutral or alkaline. Liot (*12*), Goris and Liot (*8*), and Supniewski (*21*) also have shown that acids produced by *Bacillus pyocyaneus* from the fermentation of carbohydrates may be neutralized by the ammonia produced.

In the studies by the author bromothymol blue was chosen as the indicator for use in the fermentation tests because it shows slight changes in reaction either way from neutrality. The basic medium was beef-extract broth at pH 7.0, to which was added 1 ml of a 1.6-percent alcoholic solution of bromothymol blue per liter. This medium was tubed and sterilized, after which sterile carbohydrate solutions were added aseptically. The concentrations were so adjusted that the final broth contained 0.3 percent of beef extract, 0.5 percent of peptone, and 1.0 percent of carbohydrate. After the addition of the carbohydrate solutions all tubes were tested for sterility by incubation at 32° C. for 24 hours. Each of these tubes was then inoculated with 1 drop of 24-hour-old broth culture of the test organism grown at 37°. For comparison each culture was also inoculated into a tube of broth containing no carbohydrate, and uninoculated controls of each carbohydrate medium were incubated with the culture tubes. Incubation was at 37°. Readings were taken after 1, 3, and 6 days of incubation. The 18 strains obtained from mastitis milk and 4 strains of *Pseudomonas aeruginosa* obtained from other laboratories were tested.

All of the strains were identical in their fermentation reactions. Decided or slight acid reactions were obtained in media containing arabinose, xylose, dextrose, levulose, galactose, glycerol, and mannitol. Very alkaline reactions were obtained in the medium containing no carbohydrate and in the media containing sucrose, maltose, lactose, raffinose, inulin, dextrin, and dulcitol. It may be significant that all the carbohydrates that apparently were fermented contained six carbon atoms or less and all that were not fermented, except dulcitol, contained more than six carbon atoms.

Splitting of fat tested for by the method described by Hammer and Collins (*9*), and other biochemical tests made by methods described in the Manual of Methods for Pure Culture Study (*19*), showed further characteristics common to all the strains to be: Gelatin was liquefied; milk was coagulated and proteolyzed; nitrates were reduced rapidly to nitrites and then to free nitrogen; butterfat was split; starch was not hydrolyzed; and hydrogen sulphide was not formed. All the strains from mastitis milk were motile by means of single polar flagellae. When first isolated all strains produced a chloroform-soluble blue

pigment characteristic of pyocyanin. This characteristic was lost by some strains on prolonged laboratory cultivation.

As previously stated, the ability of *Pseudomonas aeruginosa* to ferment carbohydrates and to produce indole are questions upon which various workers have failed to agree. But since all the other cultural and morphological characteristics determined for the strains in these studies, especially the ability of the freshly isolated cultures to produce pyocyanin, agree with those generally recognized as typical for *Ps. aeruginosa*, it was decided that the strains under study are members of the species *Ps. aeruginosa*.

DISCUSSION

Frobisher (*6*) states that *Pseudomonas aeruginosa*—

> probably has not the power of invading healthy tissues but sometimes gains a foothold and grows in already diseased loci, living a saprophytic existence there, possibly producing substances which result in the death and destruction of already injured cells.

That such was the case in the outbreak reported herein seems unlikely. The herd was under close observation for mastitis prior to the outbreak, and it is significant that although some streptococcic mastitis existed in the herd, most of the individuals involved in this study had had no previous history of mastitis. Two cases of acute mastitis from which coliform organisms were found in apparently pure culture occurred in the herd during the outbreak reported in this paper. In no instance was a mixed infection found and in no instance were other organisms which appeared to be significant found in any quarter either immediately before or immediately after the finding of *Ps. aeruginosa*. Although agglutination tests were inconclusive, it is concluded that *Ps. aeruginosa* was the sole etiological agent in the cases in which it was found.

From the standpoint of public health the significance of the presence of *Pseudomonas aeruginosa* in the udder is problematical; nevertheless it should not be dismissed too lightly. There are numerous references in the literature to cases of enteritis in which *Ps. aeruginosa* was considered the causative agent. Lartigau (*11*) reported outbreaks of dysentery attributed to *Bacillus pyocyaneus* in two families in which there was a total of 15 cases and 4 deaths. The source of infection in each outbreak was a contaminated well from which the family used water. In the present case the organisms were shed persistently into the milk long after the milk secretion had become apparently normal in appearance, and would be a constant source of infection to those who might be drinking raw milk from the infected animals.

The extremely active proteolysis and lipolysis exhibited by *Pseudomonas aeruginosa* makes it a factor to contend with also in the deterioration of stored dairy products. North [4] has frequently found *Ps. aeruginosa* in cream, and has observed that when initially present in relatively high numbers this organism caused spoilage at temperatures from 7° to 30° C. Stark and Scheib (*20*) found that *Ps. aeruginosa*

[4] NORTH, W. R. Personal communication. 1938.

was frequently the cause of butter deterioration. Speaking of the Gram-negative group as a whole, they state (*20, pp. 209–210*):

> Since 97 per cent (154 cultures) of these organisms hydrolyzed fat, and all of them split tributyrin, it would seem that the presence of casein-digesting gram negative rods in butter or cream might well be used as an indication of the probable spoilage of butter. Small numbers of these proteolytic gram negative rods found in fresh lightly salted, sweet cream butter, held at temperatures which would permit the growth of these organisms, was invariably followed by large numbers of the organisms and a poor quality butter.

Forty of the 154 cultures they considered in this group were *Ps. aeruginosa*.

SUMMARY

Pseudomonas aeruginosa was found to be associated with and probably the cause of an outbreak of mastitis of extreme severity. The onset was sudden and was accompanied by fever. With some cows milk production practically ceased during the acute stage of the disease. The affected quarters became blind in two cases and in others they remained hard and swollen, although secreting milk of normal appearance.

Some of the cows continued shedding the organisms in small numbers long after the acute attack had subsided and the milk had become normal in appearance.

The strains of *Pseudomonas aeruginosa* isolated from mastitis cases and four strains obtained from other laboratories differed from Bergey's description of the species in their inability to produce indole and their ability to ferment the simpler carbohydrates.

The public-health and economic significance of *Pseudomonas aeruginosa* in milk is discussed briefly.

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RELATIVE EFFICIENCY FOR GROWING LAMBS OF THE PROTEIN IN RATIONS CONTAINING ALFALFA HAY, TIMOTHY HAY, AND COMBINATIONS OF THE TWO HAYS [1]

By J. I. MILLER, *instructor in animal husbandry*, and F. B. MORRISON, *animal husbandman and animal nutritionist and head of Department of Animal Husbandry, New York (Cornell) Agricultural Experiment Station* [2]

INTRODUCTION

Few experiments have been conducted with growing animals to study specifically the nutritive value of the protein contained in common roughages. This is true even though hay is an essential part of most rations when pasture is not available. Since a considerable part of the total protein for ruminants is furnished by the hay, more data should be available as to the relative efficiency with which common roughages furnish protein in various rations.

Experiments conducted by Hart, Humphrey, and Morrison [3] showed that growing dairy heifers utilized the protein in a ration made up of corn meal, corn-gluten feed, and corn stover practically as well as the protein in a ration of alfalfa hay, alfalfa meal, and starch. The percentage of total nitrogen retained averaged 21.4 percent for the corn ration and 22.5 percent for the alfalfa ration.

Experiments conducted at the Cornell Station [4] have shown no difference in the utilization by lambs of the protein in alfalfa hay and red clover hay when fed in nutritively complete rations containing no other source of protein. On the alfalfa ration 27 percent of the total nitrogen was stored, as compared with 25 percent on the clover ration. In experiments in which corn was fed with alfalfa or clover hay in balanced rations at the same level of protein intake, 27 percent of the total nitrogen in the corn and alfalfa ration was retained, and 31 percent of the nitrogen in the corn and clover hay ration.

In another series of experiments with lambs [5] the protein in rations consisting of corn and timothy hay, supplemented with linseed meal, soybean-oil meal, or corn-gluten meal gave an average value of 21.7 for the percentage of total nitrogen stored. In other experiments lambs retained 16.4 percent of the nitrogen in rations of corn and corn stover supplemented with linseed meal, soybean-oil meal, or corn-gluten meal.

[1] Received for publication June 2, 1938.

[2] The authors acknowledge their indebtedness to J. K. Loosli of this Station for assistance in the conduct of part of the work. Grateful appreciation is also extended to Prof. L. A. Maynard, of the animal nutrition laboratory, for helpful suggestions relative to this study.

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OBJECTS OF EXPERIMENT

The high feeding value of alfalfa hay as compared with timothy hay for lamb feeding is well recognized. There are no specific data, however, to show that the protein in alfalfa has a higher nutritive value than that in timothy hay, when fed in balanced rations. In the Cornell experiments [6] [7] a ration of corn and alfalfa hay apparently furnished protein of higher nutritive value than rations of corn and timothy hay supplemented with a common protein supplement. However, these rations were fed in two separate experiments and under slightly different conditions.

To compare directly these two rations under as nearly identical conditions as possible was one object of this experiment. Another object was to compare the efficiency of the protein in rations having certain mixtures of the two hays as the roughage.

EXPERIMENTAL PROCEDURE

Nitrogen balances were determined on purebred or high-grade wether lambs of good mutton type. The urine and feces were collected daily during 10-day collection periods and all preliminary or intervening periods were 10 days or more in length. There were eight lambs used throughout the experiment.

As in preceding experiments,[8] the percentage retention of total nitrogen and of digested nitrogen was emphasized for comparing the efficiency of the protein in the rations. Biological values were calculated for the protein in the rations studied by using average values for endogenous and metabolic nitrogen as determined for lambs at this Station. The average values for the 10-day periods were 0.37 g of endogenous nitrogen per kilogram of body weight and 0.55 g of metabolic nitrogen per 100 g of dry-matter intake. The methods used in conducting the experiment were the same as those followed in the earlier work reported by this Station.

The percentage composition of the feeds used in the experiment is shown in table 1.

TABLE 1.—*Composition of feeds used*

Feed	Water	Mineral matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Alfalfa hay	8.15	5.80	14.40	1.77	31.87	38.01
Timothy hay	8.12	3.40	5.83	2.23	27.77	52.65
Corn, yellow	10.07	1.21	9.16	3.97	2.32	73.27
Soybean-oil meal	6.98	5.60	41.18	5.36	6.05	34.83

All feeds used were of good quality and were suitable for experimental work. The timothy hay was cut in early bloom and the alfalfa was also early cut. Both the timothy hay and the alfalfa hay were green and leafy. The soybean-oil meal, which was produced by the expeller process, had a cooked odor and nutlike taste. The percentage composition of the rations is given in table 2.

[6] TURK, K. L., MORRISON, F. B., and MAYNARD, L. A. See footnote 4.
[7] MILLER, J. I., MORRISON, F. B., and MAYNARD L. A. See footnote 5.
[8] MILLER, J. I., MORRISON, F. B., and MAYNARD, L. A. See footnote 5.

TABLE 2.—*Composition of the rations fed*

Feed	Alfalfa ration	Two-thirds alfalfa, one-third timothy ration	One-third alfalfa, two-thirds timothy ration	Timothy ration
	Percent	*Percent*	*Percent*	*Percent*
Alfalfa hay	50.0	33.3	16.7	
Timothy hay		16.7	33.3	50.0
Corn, yellow	30.5	46.2	44.6	40.0
Starch	17.5	2.6		
Soybean-oil meal			4.0	8.4
Corn oil	1.0			
Mineral [1]	1.0	1.2	1.4	1.6
Total	100.0	100.0	100.0	100.0

[1] The mineral mixture was composed of 40 percent of ground limestone, 40 percent of steamed bonemeal, and 20 percent of salt.

The four rations fed to the lambs contained 50 percent of roughage. The roughage in each ration was either (1) alfalfa hay alone, (2) a combination of two-thirds alfalfa and one-third timothy, (3) a combination of one-third alfalfa and two-thirds timothy, or (4) timothy hay alone. Various amounts of ground corn and a mineral supplement were included also in each ration.

The rations were prepared to contain 10 percent of total protein. Because of the differences in the amount of protein in each roughage or combinations of roughage, it was obviously impossible to prepare rations having the same amount of roughage and also the same percentage of protein in the total ration with only corn and the mineral as the other ingredients. It was, therefore, necessary to include starch in the rations with alfalfa hay or the combinations of two-thirds alfalfa and one-third timothy to bring the protein level down to 10 percent.

It was also necessary to include a protein supplement where the roughage was timothy hay or the combination of two-thirds timothy hay and one-third alfalfa hay in order to bring the protein content up to 10 percent. Soybean-oil meal was used for this purpose because it is a commonly used protein supplement and also because previous experiments at this Station,[9] [10] had shown that soybean-oil meal would furnish protein at least as efficiently as any other common protein supplement of plant source. Corn oil was included also in the alfalfa ration to equalize the energy content.

The daily allowances of feed for the 10-day periods for each lamb were weighed into individual paper bags at the beginning of the experimental periods and sampled for moisture and nitrogen determinations. These bags were stored in tightly closed metal containers. The roughage for each ration was chopped in a feed cutter, mixed with the concentrates, and fed as a part of the complete ration. The lambs were fed twice daily and the rations for each lamb were fed in a rotation to vary the order in which the lambs received the four rations.

EXPERIMENTAL RESULTS

The results of the experiment are presented in table 3 and the final values are summarized in table 4.

[9] MILLER, J. I., MORRISON, F. B., and MAYNARD, L. A. See footnote 5.

[10] TURK, KENNETH L., MORRISON, F. B., and MAYNARD, L. A. THE NUTRITIVE VALUE OF THE PROTEINS OF CORN-GLUTEN MEAL, LINSEED MEAL, AND SOYBEAN-OIL MEAL. Jour. Agr. Research 51: 401–412. 1935.

TABLE 3.—*Nitrogen-balance data showing digestibility and utilization of protein in the 4 rations* [1]

Ration and lamb No.	Body weight			Feed intake	Dry-matter intake	Nitrogen intake	Nitrogen in urine	Nitrogen in feces	Coefficient of apparent digestibility	Total nitrogen retained	Digestible nitrogen retained	Estimated biological value [2]
	Initial	Final	Average									
Alfalfa:	*Kilograms*	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	
1	28.57	29.67	29.12	8,000	7,358	129.13	56.46	46.98	63.6	19.9	31.3	63
2	23.77	24.40	24.08	8,000	7,349	128.91	53.77	50.34	61.0	19.2	31.6	62
3	35.30	36.43	35.86	9,000	8,012	141.84	71.84	50.93	64.1	13.4	21.0	57
4	32.20	32.27	32.24	9,000	7,975	140.76	65.52	56.36	60.0	13.4	22.4	58
5	39.66	41.17	40.42	9,000	8,086	143.97	83.85	48.80	66.1	7.9	11.9	51
6	35.53	36.13	35.83	9,000	8,055	151.51	78.16	50.92	66.4	14.8	22.3	55
7	34.00	34.56	34.28	8,000	7,171	134.98	63.82	46.41	65.6	18.3	27.9	60
8	23.57	24.33	23.95	7,000	6,323	112.61	44.81	34.71	69.2	29.4	42.5	68
⅔ alfalfa, ⅓ timothy:												
1	30.90	32.17	31.54	9,000	8,193	149.58	70.11	47.47	68.3	21.4	31.3	60
2	24.37	25.00	24.68	7,000	6,364	116.17	52.98	32.98	71.6	26.0	36.3	62
3	32.23	32.10	32.16	8,000	7,344	134.75	59.88	48.56	64.0	19.5	30.5	62
4	27.50	28.00	27.75	8,000	7,311	134.07	67.22	44.94	66.5	16.3	24.6	56
5	37.87	39.40	38.64	9,000	8,123	148.12	77.09	44.70	69.8	17.8	25.5	58
6	33.56	34.60	34.08	9,000	8,004	147.03	61.84	52.07	64.6	22.5	34.9	65
7	32.77	33.20	32.98	8,000	7,191	136.07	59.95	47.91	64.8	20.7	32.0	63
8	38.27	38.80	38.54	9,000	8,081	161.56	86.40	46.96	70.9	17.4	24.6	55
⅓ alfalfa, ⅔ timothy:												
1	35.37	36.53	35.95	9,000	8,193	157.91	76.16	49.38	68.7	20.5	29.8	59
2	30.47	31.33	30.90	8,000	7,227	135.11	59.05	45.58	66.3	22.6	34.0	63
3	34.47	35.80	35.14	9,000	8,181	146.02	65.96	45.38	68.9	23.8	34.5	64
4	29.67	30.00	29.84	9,000	8,235	146.85	55.14	50.07	65.9	28.4	43.0	69
5	34.47	34.93	34.70	8,000	7,411	130.86	54.31	43.80	66.5	25.0	37.6	68
6	29.63	30.40	30.02	8,000	7,420	131.00	58.27	37.62	71.3	26.8	37.6	64
7	31.13	31.56	31.34	8,000	7,209	140.01	48.99	57.06	59.2	24.3	40.9	70
8	26.60	27.37	26.98	8,000	7,118	138.42	66.57	46.20	66.6	18.5	27.8	57
Timothy:												
1	33.03	34.13	33.58	8,000	7,239	130.60	61.07	46.71	64.2	17.5	27.2	61
2	27.50	28.13	27.82	7,000	6,264	114.35	55.45	38.89	66.0	17.5	26.5	59
3	38.80	40.47	39.63	10,000	9,022	162.17	78.88	51.64	68.2	19.5	28.6	60
4	35.53	34.33	33.93	8,000	7,240	136.25	61.16	44.18	67.6	22.7	33.6	63
5	37.00	37.80	37.40	9,000	8,164	144.07	71.09	45.48	68.4	19.1	27.9	60
6	32.13	32.17	32.15	9,000	8,107	143.50	70.98	36.96	74.2	24.8	33.4	59
7	29.70	30.50	30.10	8,000	7,382	133.09	47.55	52.42	60.6	24.9	41.1	70
8	25.77	26.27	26.02	8,000	7,362	132.89	52.98	48.06	63.8	24.0	37.6	65

[1] Totals are for 10-day experimental periods.
[2] Calculated with average values for endogenous and metabolic nitrogen.

TABLE 4.—*Summary of digestibility and utilization of the protein in the rations*

Item	Data for lamb No.—								Average [1]
	1	2	3	4	5	6	7	8	
Alfalfa hay ration:									
Apparent digestibility.....percent...	63.6	61.0	64.1	60.0	66.1	66.4	65.6	69.2	64.5±0.71
Total nitrogen retained.....do....	19.9	19.2	13.4	13.4	7.9	14.8	18.3	29.4	17.0±1.51
Digestible nitrogen retained....do....	31.3	31.6	21.0	22.4	11.9	22.3	27.9	42.5	26.4±2.18
Estimated biological value.....	63	62	57	58	51	55	60	68	59 ±1.25
⅔ alfalfa, ⅓ timothy ration:									
Apparent digestibility.....percent...	68.3	71.6	64.0	66.5	69.8	64.6	64.8	70.9	67.6± .71
Total nitrogen retained.....do....	21.4	26.0	19.5	16.3	17.8	22.5	20.7	17.4	20.2± .76
Digestible nitrogen retained....do....	31.3	36.3	30.5	24.6	25.5	34.9	32.0	24.6	30.0±1.10
Estimated biological value.....	60	62	62	56	58	65	63	55	60 ± .84
⅓ alfalfa, ⅔ timothy ration:									
Apparent digestibility.....percent...	68.7	66.3	68.9	65.9	66.5	71.3	59.2	66.6	66.7± .84
Total nitrogen retained.....do....	20.5	22.6	23.8	28.4	25.0	26.8	24.3	18.5	23.7± .77
Digestible nitrogen retained....do....	29.8	34.0	34.5	43.0	37.6	37.6	40.9	27.8	35.6±1.24
Estimated biological value.....	59	63	64	69	68	64	70	57	64 ±1.11
Timothy ration:									
Apparent digestibility.....percent...	64.2	66.0	68.2	67.6	68.4	74.2	60.6	63.8	66.6± .96
Total nitrogen retained.....do....	17.5	17.5	19.5	22.7	19.1	24.8	24.9	24.0	21.3± .76
Digestible nitrogen retained....do....	27.2	26.5	28.6	33.6	27.9	33.4	41.1	37.6	32.0±1.27
Estimated biological value.....	61	59	60	63	60	59	70	65	62 ± .90

[1] The error calculated is the probable error.

Only a slight difference was found in the digestibility of the protein in the four rations. However, the alfalfa hay ration, on the average, had a slightly lower digestibility. This may have been due to the fact that more of the protein in this ration was furnished by the roughage than was true of the other rations.

The percentage of the total nitrogen retained by the lambs averaged 17.0 for the alfalfa ration, 20.2 for the two-thirds alfalfa and one-third timothy ration, 23.7 for the one-third alfalfa and two-thirds timothy ration, and 21.3 for the timothy ration. These data were analyzed statistically by the analysis of variance method proposed by Fisher, Snedecor's factor *F* being used to determine the mathematical significance.[11] A mathematical significance was found only between the data on the alfalfa ration and the ration with the mixture of one-third alfalfa and two-thirds timothy. The odds were slightly greater than 19:1. The data for the percentage of total nitrogen retained on these two rations also were analyzed by Student's method.[12] The odds were about 20:1 against this difference in the utilization of the protein in the two rations being due to chance alone.

There was a greater variation in the values obtained for individual lambs fed the alfalfa ration than for those fed the other three rations. But inspection of the data shows that five of the eight lambs had their lowest nitrogen utilization on the alfalfa ration and none gave its lowest value while on the ration with the combination of one-third alfalfa and two-thirds timothy. On the other hand, four lambs were most efficient in their use of nitrogen when fed the latter ration and only one lamb gave its highest value when fed the alfalfa ration.

While the difference in the average values for the percentage of total nitrogen retained by the lambs on these two rations is not great, the data do indicate a slight superiority in the efficiency of the protein in the ration with the mixture of one-third timothy and two-thirds alfalfa as compared with the alfalfa ration. No significant differences in the percentage of total nitrogen utilized were apparent between the means of any of the other rations.

The percentage of the digested nitrogen retained by the lambs was slightly higher on an average when the lambs were fed the ration containing the combination of one-third alfalfa and two-thirds timothy as the roughage. The average value was 35.6 percent as compared with an average of 26.4 percent for the retention of the digested nitrogen when the alfalfa ration was fed. The lambs gave intermediate values for the utilization of the digested nitrogen when fed the other two rations.

The estimated biological values averaged 59 for the ration with alfalfa hay, 60 for the ration with two-thirds alfalfa and one-third timothy, 64 for the ration with one-third alfalfa, and two-thirds timothy, and 62 for the ration containing timothy. Here again, the results indicate a slight superiority in favor of the ration with the one-third alfalfa and two-thirds timothy combination especially as compared with the alfalfa ration. The same statistical treatment of these values, however, does not indicate mathematical significance, as the odds obtained were slightly less than 19 to 1.

[11] LIVERMORE, J. R. LABORATORY EXERCISES IN STATISTICAL METHODS OF ANALYSIS. Cornell Univ. Dept. Plant Breeding. 1936.
[12] LIVERMORE, J. R. See footnote 11.

DISCUSSION

The average results from this experiment with eight lambs fed the timothy ration were almost identical with the average results of a previous experiment similarly conducted at this Station [13] on the same ration and with the same number of lambs. In the earlier experiment the average value for the percentage of total nitrogen stored was 21.2 ± 1.85 and the average for the biological values was 64 ± 1.97. Considering the variability in the utilization of the protein in this ration by different lambs and the resulting probable errors of the means, the agreement of these data with the data previously reported is remarkable.

The values obtained in this experiment for the utilization of the protein in the alfalfa ration were appreciably lower than the values obtained in an earlier experiment at this Station with lambs fed the same ration.[14] As determined with five lambs, the percentage utilization of the protein of the ration was 31 and the biological value was 77 in the previous work. The differences in the results of these two separate experiments in which practically the same rations were used are larger than would be expected. These differences may be due largely to the influence of a low-nitrogen diet fed in the earlier experiments. This period of low feed consumption as well as low nitrogen intake may have promoted increased nitrogen retention by the lambs during subsequent feeding of the experimental rations. Experiments are now under way at this Station to study this problem.

The results of the experiment reported in this paper indicate that the protein in a corn and alfalfa ration containing 10 percent of total protein is no more efficient for lambs than the protein in a comparable ration of corn, soybean-oil meal, and timothy hay. Also, that the protein in similar rations containing combinations of alfalfa and timothy hay as the roughage have little or no higher value for lambs than rations with timothy hay as the roughage. However, the conclusion cannot be drawn from this work that there is no difference in the value of the protein in alfalfa hay alone as compared with that in timothy hay alone for growing lambs, since each ration studied contained protein other than that furnished by the roughage.

Rations containing timothy hay as the only roughage have given poor results when fed to fattening lambs. In an Ohio experiment [15] timothy hay was decidedly inferior to alfalfa hay in the ration for growing, fattening lambs. A combination of equal amounts of alfalfa hay and timothy hay was but slightly inferior to alfalfa hay in the same experiment. The rations containing alfalfa hay or the combination of alfalfa hay and timothy hay were more palatable to the lambs than the ration with timothy hay as the only roughage.

[13] MILLER, J. I., MORRISON, F. B., and MAYNARD, L. A. See footnote 5.
[14] TURK, K. L., MORRISON, F. B., and MAYNARD, L. A. See footnote 4.
[15] BELL, D. S., THATCHER, L. E., and HUNT, C. H. ROUGHAGES FOR FATTENING LAMBS. Ohio Agr. Expt. Sta. Bull. 516 ((1931-32) Ann. Rept. 51): 80-81. 1933.

SUMMARY

Nitrogen-balance experiments with eight growing lambs were conducted in a study of the digestibility and utilization of the protein in the following basal rations: (1) Corn and alfalfa hay; (2) corn, a mixture of two-thirds alfalfa and one-third timothy; (3) corn, a mixture of one-third alfalfa and two-thirds timothy, and soybean-oil meal; and (4) corn, timothy hay, and soybean-oil meal. The roughage made up 50 percent by weight of the total ration, and the other ingredients were used in such amounts as were necessary to standardize each ration at the same energy and mineral content and at 10 percent total protein. All lambs were fed each of the four rations, and eight nitrogen balances of 10 days' duration were conducted on each ration.

The average percentage of total nitrogen stored was 17.0 for the alfalfa ration, 20.2 for the two-thirds alfalfa and one-third timothy ration, 23.7 for the one-third alfalfa and two-thirds timothy ration, and 21.3 for the timothy ration. The average estimated biological values were 59, 60, 64, and 62, respectively, for the four rations. There was a statistically significant difference between the values for the alfalfa ration and the one-third alfalfa and two-thirds timothy ration but between no other two rations. These data indicate that a ration of corn and timothy hay, properly supplemented, will furnish protein of as high efficiency for lambs as comparable rations containing corn and alfalfa hay.

U. S. GOVERNMENT PRINTING OFFICE: 1939